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#### ERRATA AND AUTHORS' EMENDATIONS

- Page 85, Literature Cited (2), (3), (6), "Jour. Biochem." should be "Biochem. Jour."
- Page 123, table 10, eliminate "Grams" at head of columns near top of table, and insert "Grams" over columns under "Average green weight per tree (tops and roots)"
- Page 125, table 14, column 2, "Grains" should be "Grams."
- Page 129, par. 3, line 6, "p. →" should be "(p. 114)."
- Page 210, line 6, "gross" should be "cross."
- Page 228, line 3, "identified by" should be "identified as."

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No. 1

## EFFECT OF SKIPS, OR MISSING ROW SEGMENTS, ON YIELD OF SEED COTTON IN FIELD EXPERIMENTS<sup>1</sup>

By O. A. POPE<sup>2</sup>

*Agronomist, Division of Cotton and Other Fiber Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture*

### INTRODUCTION

The effect of skips, or missing segments of row, on yield constitutes an important problem in research as well as in commercial cotton production. In field experimental work a perfect stand seldom survives, and consequently the research worker is frequently confronted with the problem of making adjustments of single-plot yields in analyzing data from field trials. If the missing row segments are due to poor germination or to disease, it may be proper to penalize the variety or treatment containing the missing segment; but if skips are due to extravarietal causes, it is logical that yields be adjusted in order that comparisons among varieties or treatments may be made with the best precision possible in the circumstances.

In the commercial production of cotton (*Gossypium* spp.), stand difficulties of two types are frequently encountered: (1) Uniformly thin stands and (2) stands with skips.

Low-germinating seed, seedling diseases, or other causes may result in a thin but rather regular stand over the field, and studies on spacing and rate of planting have shown that thin stands may give satisfactory yields under average seasonal conditions. Extensive data summarized by Brown<sup>3</sup> and Reynolds<sup>4</sup> show that substantially equal yields of

<sup>1</sup> Received for publication November 3, 1944.

<sup>2</sup> The assistance of a number of research workers was invaluable in extending the study to include a range of environmental conditions sufficiently wide to be representative of those likely to be encountered in future field work. Their co-operation is gratefully acknowledged, and the courtesy of State experiment stations in providing facilities is appreciated. The individuals and respective experiment stations are J. B. Dick, Alabama Agricultural Experiment Station; C. J. King, U. S. Cotton Field Station, Sacaton, Ariz.; G. J. Harrison, U. S. Cotton Field Station, Shafter, Calif.; J. R. Cotton, Louisiana Agricultural Experiment Station; J. W. Neely, U. S. Cotton Field Station, Stoneville, Miss.; A. R. Leding, U. S. Cotton Field Station, State College, N. Mex.; R. H. Tilley, North Carolina Agricultural Experiment Station, Statesville, N. C.; L. L. Ligon, Oklahoma Agricultural Experiment Station; D. M. Simpson, U. S. Cotton Field Station, Knoxville, Tenn.; and D. R. Hooton, U. S. Cotton Field Station, Greenville, Tex.

<sup>3</sup> BROWN, H. B. COTTON SPACING. *Miss. Agr. Expt. Sta. Bul.* 212, 16 pp. 1923.

<sup>4</sup> REYNOLDS, E. B. THE EFFECT OF SPACING ON THE YIELD OF COTTON. *Tex. Agr. Expt. Sta. Bul.* 340, 77 pp., illus. 1926.

cotton may be expected within relatively wide spacing limits at individual locations representing the major sections of the main Cotton Belt. Studies by Ware,<sup>5</sup> made at Scott, Ark., indicated that total yield was substantially the same with stands ranging from approximately 15,000 to 75,000 plants per acre, when boll weevil damage was not severe.

Replanting is seldom carried out if the stand is uniformly thin; but if fields contain many rather long skips it is customary to replant the entire row or field. In this operation most or all of the earlier plants are destroyed; frequently it is difficult for the grower to determine whether replanting is likely to be profitable, and little experimental evidence is available as a guide in deciding on the advisability of replanting.

#### EXPERIMENTAL PROCEDURE

As a consequence of the need for information on the effect of missing row segments on experimental procedure and in practical production, a series of experiments was arranged in 1936 to study this problem. The plan, used at nine locations in 1936 and 1937, at seven locations in 1938, and at two locations in 1939, consisted of a 10 by 10 Latin square including a perfect-stand check and artificial skips ranging from 2 to 10 feet in increments of 1 foot. Three-row plots 25 feet long were used throughout the study, and skips were made in the center row at the normal thinning date. The use of a uniform plot size and a similar plan at all locations greatly facilitated the statistical treatment of the data. Several of the cooperating stations obtained data for 3 years; but other stations were prevented from full participation by the fact that a perfect stand over the entire area was not obtained, and consequently the desired pattern of skips could not be produced. In most cases the skip-correction studies were carried out on multiplication blocks or general plantings, and for this reason different blocks of land were used in succeeding years at several of the stations. Different varieties or strains were used at various locations, and in some cases different strains were used in succeeding years at the same location. Combined analyses were not made either for single years or for the whole study, since year and place effects would have been confounded with variety or strain and also with experimental area within locations.

The questions of greatest practical importance are the effect of skips on total plot yield and the distribution of this effect among rows within the plot. The minimum amount of data satisfactory for analysis consisted of the yield of each plot by individual rows. In order that the distribution of effect of skips of different lengths within the plot might be determined, data were obtained on either boll counts or weight of seed cotton by row segments as indicated in the following plan:

<i>Left segments</i>	<i>Skip length</i>	<i>Right segments</i>
Row <i>a</i> , 3-L 2-L 1-L	adjacent	1-R 2-R 3-R
Row <i>b</i> , 3-L 2-L 1-L	skip	1-R 2-R 3-R
Row <i>c</i> , 3-L 2-L 1-L	adjacent	1-R 2-R 3-R

Within each plot the rows were designated *a*, *b*, and *c*. In each of the outside rows *a* and *c* a section opposite the skip and of the same

<sup>5</sup> WARE, J. O. COTTON SPACING. I. STUDIES OF THE EFFECT ON YIELD AND EARLINESS. *Ark. Agr. Expt. Sta. Bul.* 230, 84 pp. 1929.

length as the skip was designated "adjacent." Within each row the two sections contiguous to the skip of row *b* or to the adjacent parts of the outside rows were designated for the segment studies as 1-L, 2-L, or 3-L (left) and 1-R, 2-R, or 3-R (right). Each L or R segment was 3 feet long. Data on boll counts or seed-cotton weights for the various segments were recorded in the proper location on plot sketches. In most tables reporting of only two decimal places for mean actual yields and of only one for mean percentage yields seemed sufficient to show differences.

### EXPERIMENTAL RESULTS

Data from the skip-correction study consisted of yield of seed cotton by single rows and boll counts or weights on the "adjacent" and 3-foot segments outlined in the plot plan previously discussed.

#### YIELD ON TOTAL PLOT

Total-plot yields, in pounds of seed cotton, for the 9 participating locations in 1936, are summarized in the first 11 columns of table 1. Plot size was the same throughout the study, but variations in average yield were rather wide as is evident from the range in station means, 1.23 to 15.35 pounds. It is noticeable, however, that the yields for various skip lengths within any location tend to be consistent, and there was no marked reduction in yield with increasing skip length at any single location or in the average for all locations.

Because of wide differences in average yield among stations, it seemed desirable to convert actual yield to a percentage of each station average for ease in comparing tests. The percentage yields for each station are shown in the last 10 columns of table 1, and these confirm the actual yields in showing that there was no marked reduction in yield with increasing skip length. The averages of all stations indicate that in 1936 there was a slight tendency for the longer skips to produce smaller yields; but it will be noted that the 10-foot skip was accompanied by only a small reduction in yield, although it represented a 13.3-percent reduction in the area of the plot.

Summaries of the yields of seed cotton for whole plots in the 1937, 1938, and 1939 studies also are shown in table 1. These studies, which included data from some additional stations and from some of the same stations in succeeding years, confirmed the findings in 1936 that skips in an interior row of a multiple-row plot have little effect on total yield of the plot. A tendency for a slight reduction in yield on the plots having greater skip lengths is evident in the averages for all stations in each of the years. The proportionate decrease in yield with increasing skip length is not consistent at all stations and not significant in any of the 27 tests included in the study.

Analyses of variance, according to the methods developed by Fisher,<sup>6</sup> were made on whole-plot yields for each location in each year, and a summary of these analyses is shown in table 2. Wide differences were found in mean squares for total variance and for the variance due to columns, rows, and error, and these findings reflect the hetero-

<sup>6</sup> FISHER, R. A. STATISTICAL METHODS FOR RESEARCH WORKERS. Ed. 6, rev. and enl., 339 pp., illus. London. 1936.

TABLE 1.—*Total yields of seed cotton at various locations, 1926–39*

Year and location	Pounds per plot with indicated skip length (feet)										Percentage of station average yield with indicated skip length (feet)											
	0	2	3	4	5	6	7	8	9	10	Average	0	2	3	4	5	6	7	8	9	10	
<b>1936</b>																						
Alabama	2.73	3.08	3.10	2.81	2.85	2.70	2.89	2.72	2.68	2.65	2.83	96.5	108.8	109.6	99.4	100.5	98.6	102.0	96.0	94.8	93.7	
California	15.73	16.65	15.18	15.52	14.10	15.76	15.99	14.50	15.80	15.30	15.35	102.5	101.9	98.9	101.1	91.8	102.6	104.1	104.1	102.9	99.6	
Louisiana	7.22	7.24	7.08	7.79	7.48	7.16	6.87	6.75	6.72	6.75	7.17	100.8	101.1	98.8	108.7	95.9	104.4	99.1	95.3	100.7	94.2	
Mississippi	7.89	6.94	7.28	7.01	6.92	6.63	7.43	7.46	7.54	7.76	7.19	106.8	105.6	101.3	97.5	96.3	92.3	103.4	103.8	104.9	94.1	
New Mexico	10.32	10.69	10.68	9.67	10.69	10.27	10.24	9.91	9.38	9.84	10.16	101.6	104.9	105.1	95.2	99.3	101.1	100.8	97.5	97.7	96.8	
North Carolina	5.14	5.15	5.20	5.09	4.99	5.26	5.17	5.15	5.12	5.24	5.16	99.5	99.8	102.4	100.1	98.6	96.5	102.4	100.1	99.1	101.5	
Oklahoma	1.80	1.23	1.22	1.20	1.25	1.26	1.21	1.24	1.19	1.19	1.19	1.23	105.5	100.3	99.6	97.6	102.0	102.8	100.8	99.6	97.2	97.3
Tennessee	7.94	8.21	8.21	8.00	8.25	7.73	8.15	8.03	8.11	8.03	8.11	97.9	101.2	104.3	100.5	101.1	98.6	101.7	95.3	100.4	90.0	
Texas	4.34	4.28	4.12	4.34	4.16	4.08	4.21	4.21	4.26	4.06	4.21	103.2	101.8	98.0	103.2	99.1	107.0	100.1	101.0	96.5	101.0	
Average	6.96	6.94	6.94	6.84	6.84	6.60	6.84	6.95	6.64	6.88	6.65	6.82	101.9	101.7	101.7	101.7	100.3	96.8	100.2	101.8	97.3	
<b>1937</b>																						
Arizona (Mesa)	10.85	11.02	11.65	10.73	11.19	10.73	10.96	10.29	11.15	10.87	10.33	11.05	99.0	104.7	104.4	104.4	102.3	98.6	102.8	98.6	100.7	
Arizona (Sacaton)	10.86	11.64	11.49	10.57	11.31	10.63	10.01	11.82	10.87	10.84	10.51	100.4	101.4	102.6	102.6	98.6	102.9	92.9	100.5	106.9		
California	18.31	18.51	18.52	17.79	17.86	17.18	18.4	19.08	17.84	17.60	18.04	101.5	102.6	98.6	102.6	100.5	106.7	98.7	98.9	93.5		
Louisiana	11.77	12.34	11.51	11.82	12.33	12.18	11.82	11.64	11.50	11.79	109.8	101.9	107.7	100.7	104.6	103.3	97.7	95.7	98.7	97.6		
Mississippi	11.44	12.27	12.09	12.46	11.86	11.56	11.77	11.65	11.72	11.74	11.86	103.5	103.5	105.0	105.0	104.0	100.7	98.2	98.8	99.0		
North Carolina	8.69	8.85	8.90	8.59	8.46	8.39	8.53	8.53	8.54	8.33	8.11	8.63	100.8	102.6	103.9	103.5	98.0	108.8	98.8	99.0	94.0	
Oklahoma	2.71	2.61	2.68	2.46	2.68	2.81	2.60	2.62	2.68	2.67	2.65	92.6	98.4	102.6	102.6	100.9	105.8	97.9	99.6	101.2		
Tennessee	4.68	4.73	4.78	4.97	4.65	4.74	4.68	4.82	4.63	4.47	4.75	98.6	100.8	104.7	102.2	99.0	98.5	101.6	97.6	96.4		
Texas	4.80	4.88	4.72	4.91	4.77	4.76	4.76	4.91	4.81	4.71	4.30	93.3	101.3	101.1	97.8	98.8	101.7	99.7	97.6	101.5		
Average	9.37	9.64	9.59	9.48	9.48	9.28	9.23	9.44	9.18	9.17	9.38	99.8	101.9	101.7	100.6	100.9	100.1	98.3	100.0	97.8	98.0	
<b>1938</b>																						
Arizona (Mesa)	12.71	12.79	13.02	12.44	12.61	12.54	12.10	12.32	11.86	12.26	12.46	102.0	102.6	104.4	99.8	101.2	100.6	97.1	98.8	95.1		
Arizona (Sacaton)	12.17	12.17	12.16	11.33	12.10	11.73	11.14	11.11	11.54	12.10	11.76	103.5	103.5	103.3	96.3	103.6	99.7	94.7	94.5	98.1		
California	14.62	15.38	14.55	15.09	14.45	15.09	14.84	14.32	14.20	14.20	14.78	98.5	104.1	97.8	102.1	102.7	100.4	100.8	102.6	102.8		
Mississippi	10.07	9.80	9.74	9.86	9.56	9.58	9.47	9.89	9.80	9.85	10.00	9.84	102.3	98.6	100.2	100.5	100.5	96.2	100.5	101.6		
North Carolina	7.91	7.93	7.61	7.84	8.04	7.81	7.34	7.88	7.76	7.76	7.76	101.8	97.7	100.6	103.2	100.2	94.3	101.2	99.9	99.6		
Tennessee	4.00	4.23	3.96	4.40	4.35	4.20	3.89	4.31	4.36	3.92	4.16	96.2	101.6	95.2	105.9	104.6	100.9	93.4	103.5	104.3		
Texas	4.74	4.88	4.93	4.54	4.87	4.73	4.36	4.47	4.47	4.33	4.60	103.0	99.5	107.1	98.6	105.8	102.8	94.7	97.1	94.1		
Average	9.46	9.55	9.41	9.36	9.69	9.33	9.03	9.18	9.15	9.31	9.34	101.1	101.8	100.6	103.1	100.1	96.5	98.9	98.7	98.7		
<b>1939</b>																						
Arizona (Mesa)	14.70	14.48	14.77	14.65	14.58	13.91	14.15	14.09	14.19	14.19	14.37	102.3	100.8	102.8	101.9	101.4	96.8	98.5	98.0	98.7		
New Mexico	13.89	13.74	13.33	13.12	13.70	12.71	13.53	13.12	13.43	13.55	13.41	103.6	102.4	99.4	97.8	102.1	94.8	100.9	97.8	101.0		
Average	14.29	14.11	14.05	13.88	14.14	13.31	13.84	13.60	13.81	13.87	13.89	102.9	101.6	101.1	99.9	101.8	95.8	99.7	97.9	99.4	99.9	
Average, 1936–39	8.95	9.05	8.90	8.89	8.89	8.78	8.78	8.75	8.75	8.75	8.75	97.1	98.6	101.1	102.2	101.5	100.5	103.4	98.8	98.4		

geneity of plant response due to location and among individual plots within each experimental block. The one consistent and important result of these analyses is that the contribution for skips was not significantly different from error in any of the 27 tests. The conclusion that skip lengths of as much as 10 feet in an interior row of a multiple-row plot have no significant effect on total-plot yield seems to be well substantiated.

TABLE 2.—*Analyses of variance for total yield of seed cotton in table 1*

Year and location	Mean squares <sup>1</sup>				
	Total	Columns	Rows	Skips	Error
<i>1936</i>					
Alabama	0.2778	0.9306	0.6811	0.2409	0.1504
California	17.5835	128.3349	38.6971	3.7331	2.8317
Louisiana	1.8092	13.2777	.6330	.9383	.6315
Mississippi	1.7655	2.2920	8.8943	1.5747	.8324
New Mexico	2.5127	3.0270	16.6183	1.1414	.8566
North Carolina	.6196	1.7041	2.3645	.0901	.3320
Oklahoma	.0195	.0149	.0697	.0122	.0147
Tennessee	2.8019	23.4720	3.2158	.3976	.4670
Texas	.1927	.8149	.3100	.0902	.1119
<i>1937</i>					
Arizona (Mesa)	2.5269	12.3358	1.3472	1.6996	1.5516
Arizona (Sacaton)	6.8888	12.6771	31.7891	3.5349	3.4719
California	3.2711	9.1359	3.4182	3.9524	2.4345
Louisiana	2.6541	8.9535	5.8539	1.4061	1.6226
Mississippi	2.9732	18.8689	2.4514	1.0372	1.2935
North Carolina	1.6741	10.4184	1.6187	1.3062	.6339
Oklahoma	.2021	.6503	.4755	.0803	.1260
Tennessee	.7170	.9523	3.5669	.1369	.4042
Texas	.1413	.2865	.4532	.0656	.0937
<i>1938</i>					
Arizona (Mesa)	1.1530	4.1192	1.3016	1.1814	.7601
Arizona (Sacaton)	1.9125	2.1033	-----	1.4194	1.9734
California	3.3159	10.8326	3.9615	1.4633	2.5271
Mississippi	.9283	3.1698	2.6962	.2587	.5109
North Carolina	.4721	.8662	1.0489	.3788	.3626
Tennessee	.4969	1.0811	1.5304	.3944	.3068
Texas	.2244	.1735	.9675	.4295	.1123
<i>1939</i>					
Arizona (Mesa)	1.6003	8.4939	4.1116	.8963	.5126
New Mexico	1.2655	6.9617	1.0435	1.2471	.5335

<sup>1</sup> Degrees of freedom: 99 for total; 9 for columns, rows, and skips; and 72 for error.

## YIELD ON MIDDLE ROW

A summary of the yields of seed cotton on middle rows for the 9 locations in 1936 is shown in the first 11 figure columns of table 3. The general level of yields varied widely among locations, the station averages ranging from 0.36 to 4.44 pounds. In order that comparisons might be made more easily, actual yields were converted to percentages of each station average, and these means are tabulated for each station in the last 10 columns of table 3. On either the actual or the percentage basis, there is a substantially linear decrease in yield of seed cotton with increasing skip lengths for each test and for the average of all locations. The tendency for reduction in yield appears to be reasonably consistent despite wide differences in general level of yield. The extremely low yields in Oklahoma are a result of severe drought, but the decrease in yield on middle row for increasing skip lengths at this station was reasonably consistent with other localities in the rain belt and also with the 2 stations, California and New Mexico, where cotton was produced under irrigation. This consistency in behavior suggests that the trends exhibited represent a general

TABLE 3.—*Yields of seed cotton on middle rows at various locations, 1938-39*

Year and location	Pounds per middle row with indicated skip length (feet)										Percentage of station average yield of middle row with indicated skip length (feet)										
	0	2	3	4	5	6	7	8	9	10	Average	0	2	3	4	5	6	7	8	9	10
1938																					
Alabama	0.86	0.96	0.88	0.87	0.85	0.79	0.70	0.72	0.63	0.83	104.0	116.8	119.2	107.1	105.6	102.9	95.7	85.3	86.9	76.4	
California	6.12	5.11	4.81	4.70	4.21	4.43	4.55	3.81	3.99	3.64	4.44	115.4	115.2	105.9	105.9	102.5	99.8	85.9	89.9	82.0	
Louisiana	2.43	2.22	2.35	2.37	2.18	2.01	2.21	2.18	2.07	1.82	2.22	109.3	107.9	105.5	112.8	97.1	107.5	95.6	88.2	93.1	81.8
Mississippi	2.69	2.22	2.38	2.37	2.18	2.01	2.21	2.18	1.86	2.23	107.7	99.6	120.7	106.8	100.4	102.5	90.2	97.8	83.3	85.7	
New Mexico	3.64	3.59	3.50	3.01	3.02	2.94	2.64	2.58	2.47	2.17	2.05	107.6	121.9	118.8	102.2	102.5	99.8	86.6	87.6	83.7	73.7
North Carolina	1.68	1.87	1.75	1.74	1.68	1.65	1.62	1.49	1.40	1.33	1.33	1.56	104.0	104.4	103.0	104.0	95.9	90.2	85.4	85.2	80.2
Oklahoma	1.44	1.38	1.36	1.36	1.40	1.40	1.36	1.36	1.36	1.36	1.36	106.5	120.0	112.2	106.5	112.1	99.5	100.9	93.4	88.1	83.9
Tennessee	2.68	2.72	2.78	2.72	2.69	2.64	2.31	2.39	2.16	2.30	2.10	109.0	110.0	112.2	106.5	103.0	104.4	104.1	96.9	87.8	85.4
Texas	1.42	1.35	1.27	1.32	1.20	1.24	1.21	1.12	1.09	1.24	1.14	108.5	102.1	106.1	103.7	113.0	103.1	103.1	90.0	90.6	87.6
Average	2.29	2.25	2.17	2.01	2.02	1.97	1.81	1.82	1.66	2.03	114.1	112.7	110.6	106.7	109.1	99.3	97.0	89.0	89.8	81.6	
1937																					
Arizona (Mesa)	3.76	3.79	3.69	3.46	3.19	3.17	3.22	2.66	2.47	2.71	3.21	117.1	118.0	114.9	107.7	99.3	98.7	100.2	82.8	76.9	84.4
Arizona (Sacaton)	3.63	3.55	3.53	3.70	3.59	5.31	4.85	2.92	2.87	3.10	2.62	2.51	3.21	110.7	111.6	115.6	111.9	96.7	80.5	81.7	78.3
California	5.98	5.97	5.99	5.42	5.31	4.85	4.89	4.91	4.35	4.03	5.17	115.6	115.9	115.5	115.5	102.7	94.6	95.0	84.1	81.7	87.3
Louisiana	3.91	3.91	3.72	3.95	4.00	3.78	3.78	3.42	3.49	3.30	3.73	104.7	113.0	99.5	103.5	107.2	101.2	97.5	91.6	91.1	88.5
Mississippi	3.77	3.96	3.97	3.95	3.72	3.95	3.86	3.56	3.48	3.52	3.97	3.13	3.62	104.4	109.5	109.8	109.2	106.7	98.4	96.2	97.3
North Carolina	2.94	2.79	2.89	2.82	2.57	2.81	2.36	2.39	2.20	2.09	2.59	108.0	111.7	108.6	108.8	102.5	92.5	95.3	85.3	80.9	
Oklahoma	1.95	1.83	1.96	1.76	1.80	1.76	1.76	1.73	1.68	1.68	1.68	119.8	104.3	101.9	101.9	94.9	91.7	95.9	84.8	81.8	
Tennessee	1.62	1.58	1.46	1.65	1.45	1.35	1.25	1.32	1.17	1.18	1.40	115.6	112.5	104.3	117.3	103.5	96.1	93.3	90.4	83.0	
Texas	1.62	1.57	1.59	1.47	1.41	1.37	1.36	1.26	1.27	1.46	116.3	108.8	107.4	107.4	108.5	96.4	93.7	93.0	86.2	86.9	
Average	3.14	3.15	3.09	3.03	2.92	2.74	2.65	2.60	2.35	2.32	2.80	113.4	111.4	110.6	108.2	103.5	98.5	94.1	92.7	84.0	83.6
1938																					
Arizona (Mesa)	4.36	4.19	4.21	3.92	3.83	3.66	3.54	3.33	3.01	3.21	3.73	117.0	112.4	113.0	105.2	102.8	98.2	95.0	89.4	80.8	86.1
Arizona (Sacaton)	4.92	4.46	4.39	3.93	4.00	3.76	3.54	3.36	3.44	3.31	3.83	109.4	114.2	102.3	104.2	97.8	92.3	97.4	89.7	86.3	
California	3.39	5.06	4.68	4.66	4.66	4.37	4.50	4.14	4.01	3.83	3.70	4.42	111.4	114.6	105.9	106.5	102.5	99.4	90.8	86.7	83.8
Mississippi	3.39	3.16	3.21	3.36	3.38	3.36	3.48	3.32	3.29	3.20	3.30	108.6	101.7	103.8	108.1	108.5	108.5	99.8	95.3	86.9	87.5
North Carolina	2.61	2.60	2.53	2.52	2.52	2.28	2.14	2.11	2.19	2.04	2.36	110.3	113.7	107.7	106.8	106.8	106.8	96.7	92.6	86.2	86.2
Tennessee	1.48	1.50	1.33	1.32	1.33	1.19	1.13	1.23	1.17	1.08	1.28	115.6	117.0	103.9	103.1	107.6	93.3	88.3	91.7	84.1	
Texas	1.56	1.53	1.54	1.44	1.30	1.37	1.20	1.22	1.17	1.05	1.36	114.9	112.7	113.4	106.0	110.5	100.9	88.4	80.8	77.3	
Average	3.22	3.23	3.13	3.02	3.04	2.84	2.68	2.60	2.50	2.44	2.87	112.5	112.6	108.7	105.3	106.5	98.4	92.5	91.1	87.8	84.5
1939																					
Arizona (Mesa)	4.96	4.92	4.73	4.51	4.20	4.18	3.78	3.82	3.79	4.38	113.2	112.3	108.5	102.9	95.9	95.4	86.8	87.2	86.6	86.6	
New Mexico	4.50	4.38	4.34	3.97	4.00	3.63	3.73	3.36	3.26	3.09	3.83	117.3	114.2	113.5	106.5	106.6	94.6	97.3	87.6	85.0	80.6
Average	4.73	4.65	4.63	4.35	4.30	3.91	3.95	3.57	3.54	3.44	4.11	115.3	113.2	112.7	105.7	104.8	95.3	96.3	86.9	86.1	83.5
Average, 1936-39	3.00	3.00	2.93	2.84	2.75	2.61	2.53	2.41	2.30	2.22	2.66	113.0	112.7	110.3	103.8	103.4	98.2	95.1	90.6	86.5	83.5

relation that may be expected to hold under a wide range of growth conditions.

Mean data for yield of seed cotton on the middle rows in 1937, 1938, and 1939 also are shown in table 3. The trend in mean values for individual stations on the basis of either actual weights or percentages of station averages is closely similar to the trends exhibited in the 1936 data and for this reason requires little detailed comment.

A summary of analyses of variance by individual locations and years for yield on middle row in pounds is shown in table 4. The mean squares for total variance and for columns and rows vary widely with locations and seasons, owing to differential plant response to soil heterogeneity and to prevailing weather conditions. Mean-square variances for skips and error differ likewise with locations and seasons, but the variance due to skips was significantly greater than error in each of the 27 tests. This consistency establishes the fact that differences in skip length are definitely related to yield of the row in which the skip occurs.

TABLE 4.—*Analyses of variance for middle-row yields of seed cotton in table 3*

Year and location	Mean squares <sup>1</sup>				
	Total	Columns	Rows	Skips	Error
<i>1936</i>					
Alabama.....	0.0432	0.0695	0.0570	0.1305	0.0272
California.....	1.9415	11.8040	4.3598	2.6891	.3093
Louisiana.....	.2464	1.3749	.0610	.5054	.0962
Mississippi.....	.2457	.2611	.0211	.4967	.1279
New Mexico.....	.4904	.3493	1.4734	2.3783	.1498
North Carolina.....	.1238	.2396	.3231	.3403	.0574
Oklahoma.....	.0064	.0037	.0141	.0241	.0036
Tennessee.....	.3561	2.1845	.5189	.6454	.0710
Texas.....	.0520	.0718	.1371	.1156	.0309
<i>1937</i>					
Arizona (Mesa).....	.7239	1.2953	1.6971	2.2600	.3388
Arizona (Sacaton).....	.9297	1.3207	2.5111	2.0702	.5406
California.....	.8841	1.0727	.5691	4.7456	.4173
Louisiana.....	.3720	.7819	.4259	.8725	.2515
Mississippi.....	.7603	2.2131	2.4962	1.2476	.3132
North Carolina.....	.2726	1.2142	.2124	.9361	.0796
Oklahoma.....	.0336	.0494	.0453	.0970	.0222
Tennessee.....	.1387	.2375	.3902	.3133	.0731
Texas.....	.0482	.0488	.0648	.2233	.0231
<i>1939</i>					
Arizona (Mesa).....	.4225	.4927	.4454	2.0829	.2034
Arizona (Sacaton).....	.4894	.1247	-----	1.2558	.4022
California.....	.6101	1.1604	.6218	2.1816	.3434
Mississippi.....	.1958	.2362	.2298	.6265	.1327
North Carolina.....	.1404	.1057	.2057	.5540	.0848
Tennessee.....	.1058	.1037	.1751	.2050	.0849
Texas.....	.0511	.0140	.0686	.3386	.0177
Arizona (Mesa).....	.4038	.6032	.4759	2.3923	.1213
New Mexico.....	.3956	.6244	.1800	2.4911	.1321

<sup>1</sup> Degrees of freedom: 99 for total; 9 for columns, rows, and skips; and 72 for error.

Significance was not established in any test for total-plot yield, but significance in yield on middle row was established in every test; consequently it is clear that some lateral compensation occurred in sections of the outside rows adjacent to the middle-row skips. In 1936 the average yield of outside rows was analyzed to test the gain of outside rows due to the presence of skips in the middle row. A gain in yield for outside rows adjacent to long skips over those adjacent to short skips or the perfect-stand check was evident at all

nine locations. At three locations variance for skip length exceeded error at odds of 19:1, but at six locations no significance was indicated. Since a trend was evident in all cases but was usually nonsignificant, the conclusion that gain in yield for rows adjacent to skips is generally not greatly different from error seems justified. Since total-plot yield and yield of middle rows indicated the relation adequately, separate calculations on the outside rows were not made in the remainder of the study.

#### BOLL COUNTS AND YIELD ON 3-FOOT SEGMENT OF ROW

Boll counts were made on the first, second, and third 3-foot segments by rows on each plot as shown in the plan on page 2. These boll counts may be expected to measure the yield on plants adjacent to skips relative to those on plants occurring far enough away to be unaffected, and in this way the extent to which compensation occurs along the two parts of the middle row may be estimated. Boll counts on the third 3-foot segments (3-L and 3-R) could not be obtained on some of the 9- and 10-foot-skip plots because of the size of plot, and for this reason complete analyses of variance were limited to the first and second 3-foot segments.

Rather complete studies were made on all segment relations in the 1936 data. Averages of 3-L and 3-R, when these data were available, were compared with averages of 2-L and 2-R within each experiment, but no consistent differences were apparent; this indicates that compensation along the row does not generally extend beyond plants in the first 3-foot segment adjacent to the skip.

Lateral comparisons between 2-L, 3-L, 2-R, and 3-R segments revealed no consistent differences between row *b* and rows *a* and *c*. Likewise comparisons between 1-L and 2-L and 1-R and 2-R within rows *a* and *c* failed to show consistent trends. Consequently it is clear that compensation within a multiple-row plot occurs largely, if not entirely, on plants within 3 feet of the skip ends in the row having the skip and on plants in the adjacent segments of rows contiguous to the skip.

After the pattern of compensation within plots was established, analyses of data were continued only on those segments affected by skips and in this report only the minimum number of variables capable of adequately presenting the complete relations are included.

Boll counts were obtained at five locations in 1936, four locations in 1937, five locations in 1938, and one location in 1939. Summaries by locations for segments in the middle row for each skip length are given in table 5.

No significant differences occur between first and second 3-foot segments for the no-skip checks, but distinct differences are evident between the first and second segments for all skip lengths of 2 feet and greater. There is some tendency for the first segment on the longer skips to have slightly higher boll counts than on the shorter skips, but this trend is less evident at individual locations than in seasonal averages. The absence of any pronounced and consistent increase in boll counts on the first segment for increasing skip lengths suggests that a linear correction may be expected to apply reasonably well in making adjustments for missing segments in experimental work.

TABLE 5.—*Boll counts on middle rows at various locations, 1936–39*

Year and location	Boll counts for specified skip lengths and 3-foot segments																
	None		2 feet		3 feet		4 feet		5 feet		6 feet		7 feet		8 feet		
	1st segment	2d segment	1st segment	2d segment	1st segment	2d segment	1st segment	2d segment	1st segment	2d segment	1st segment	2d segment	1st segment	2d segment	1st segment	2d segment	
1936																	
New Mexico	67.9	73.6	77.7	80.8	82.2	87.8	65.6	78.5	68.6	75.7	71.2	74.4	67.1	72.2	65.0	74.7	
North Carolina	42.3	46.4	52.6	43.2	54.3	57.6	43.5	54.7	38.8	57.8	41.1	62.3	39.2	57.6	43.4	60.6	44.1
Oklahoma																	55.6
Tennessee	58.6	57.8	74.3	51.0	17.8	20.8	17.2	24.2	19.9	23.7	21.9	23.7	18.9	22.8	17.7	26.4	23.8
Texas	37.1	41.0	48.8	34.4	71.5	51.6	77.9	62.0	76.1	56.1	73.6	70.5	63.3	80.4	56.7	80.4	75.3
Average	51.5	54.7	55.6	44.9	55.4	44.9	58.7	46.3	57.7	44.8	55.9	46.5	57.4	43.7	59.6	43.2	57.3
1937																	
Mississippi	87.7	87.5	110.1	86.5	115.9	89.2	113.5	85.7	141.6	91.3	128.5	85.7	131.5	86.1	120.6	93.7	127.9
North Carolina	46.8	49.1	55.6	42.9	57.0	46.2	52.3	49.4	60.8	46.5	72.2	49.7	66.0	44.8	68.7	51.7	67.1
Tennessee	47.2	41.6	49.6	41.2	35.0	62.0	42.7	66.3	39.8	55.9	42.1	55.9	42.1	70.0	46.2	62.1	61.9
Texas	53.3	50.3	60.3	41.3	60.5	50.6	72.0	40.3	69.0	47.4	65.9	45.1	64.8	47.0	60.5	47.4	73.2
Average	56.9	57.9	63.9	51.3	71.9	55.2	77.5	54.5	81.9	56.0	80.6	54.9	82.1	54.2	79.9	59.7	81.1
1938																	
Mississippi	65.5	71.5	90.2	70.1	88.0	70.6	102.3	66.4	103.2	71.7	109.3	69.3	107.2	71.2	117.7	74.5	110.1
North Carolina	46.7	43.8	53.4	48.8	59.7	44.3	56.6	47.1	63.5	43.3	58.7	43.5	58.7	37.8	59.2	44.3	57.6
Oklahoma																	
Tennessee	37.6	34.2	41.9	38.8	75.2	61.7	72.7	64.5	77.4	64.4	88.6	57.4	85.1	60.4	80.5	57.4	79.4
Texas	34.0	33.1	42.1	33.4	32.8	43.2	28.8	30.3	44.2	30.9	40.6	31.2	43.3	28.3	45.3	32.9	43.0
Average	45.9	45.6	50.9	48.6	62.5	47.5	63.6	48.4	67.4	48.3	69.0	47.1	67.7	46.0	69.8	48.5	70.7
1939																	
New Mexico	77.8	76.4	78.2	73.8	81.4	74.7	81.7	71.5	87.4	69.1	87.8	71.7	87.7	76.5	86.9	73.0	88.5
Average, 1936–39	83.5	54.6	62.1	49.8	63.9	50.5	66.9	50.9	69.4	50.5	69.0	50.6	69.4	49.4	70.2	51.4	69.5

At Baton Rouge, La., weights of seed cotton instead of boll counts were determined for 3-foot segments, and summaries of these yields are shown in table 6. In 1936 an increase in yield for the first 3-foot segment is evident for the 2-foot skip length, and there is some evidence of a progressive increase with increasing skip lengths. In 1937 there is somewhat stronger evidence of an association between yield of the first 3-foot segment and increasing skip length, indicating that in this test somewhat greater compensation occurred in those plots containing longer skips than in those with shorter ones.

TABLE 6.—*Yield of seed cotton in pounds on 3-foot segments of middle row in Louisiana, 1936 and 1937*

Skip length (feet)	1936		1937		Average	
	1st segment	2d segment	1st segment	2d segment	1st segment	2d segment
0.....	0.315	0.302	0.449	0.472	0.382	0.387
2.....	.371	.300	.636	.545	.503	.422
3.....	.413	.274	.672	.498	.542	.386
4.....	.490	.324	.749	.545	.619	.434
5.....	.466	.253	.513	.546	.640	.399
6.....	.505	.338	.787	.566	.646	.452
7.....	.467	.297	.888	.486	.667	.391
8.....	.438	.274	.823	.504	.630	.389
9.....	.524	.354	.956	.480	.740	.417
10.....	.450	.297	.923	.510	.686	.403
Average .....	.444	.301	.768	.515	.606	.408

A summary of the analyses of variance for boll counts on first and second 3-foot segments in the middle row is shown in table 7. A comparison of mean squares among locations indicates wide differences in heterogeneity of data among the various places. This is reflected not only in total variance but also in all components of the whole-plot and split-plot portions of the analyses. However, comparisons within any one station are valid, even though combined analyses might be of questionable value. The whole-plot portion of the analyses measures the total effect of boll counts or seed-cotton weights for the sum of the first and second 3-foot segments. It is not surprising, therefore, that at many locations skip length fails to exceed error (a) significantly, since the mean values indicate that the major differences are between the first and second 3-foot segments.

In the split-plot portion of the analyses variance due to positions measures the average difference between the first and second 3-foot segments and that due to positions  $\times$  skips measures the differential in boll counts or weight of seed cotton among the various skip lengths. At every location the variance due to positions significantly exceeds not only error (b) but positions  $\times$  skips as well, which indicates that the differences between the first and second segments are unquestionably significant and that these differences are largely independent of the skip length. These findings indicate that a linear correction is generally satisfactory in making adjustments for skips, or missing segments, in field experimental work.

TABLE 7.—Analyses of variance for boll counts in table 5 and for yields of seed cotton in table 6

## BOLL COUNTS OF TABLE 5

Year and location	Mean squares <sup>1</sup>						Total Error (b)	
	Whole plot			Split plot				
	Rows	Columns	Skips	Error (a)	Positions	Positions × skips		
1936								
New Mexico	10.5302	1.8070	1.0829	1.2068	30.8725	2.2874	0.8087	
North Carolina	3.2834	2.8312	1.0269	.7799	81.6642	2.5039	.2746	
Oklahoma		4.1514	3.5115	.2799	20.11.4005	1.1452	.0046	
Tennessee (Knoxville)	305.3539	1.692.4450	234.0561	147.6131	403.0096	229.0083	368.7606	
Texas (Greenville)	151.9467	88.3889	142.5356	69.8869	3.511.2200	88.4200	118.1583	
1937								
Mississippi	3.694.1467	3.456.6356	1.280.2356	647.7522	55.112.0000	1.162.2222	376.3778	
North Carolina	220.3276	11.4036	3.0015	.9123	108.0450	2.5573	.4035	
Tennessee (Knoxville)	220.3878	437.7122	587.7766	217.3325	18.339.6200	242.3089	281.7311	
Texas (Greenville)	242.7806	149.3101	148.1717	107.3314	16.900.6050	659.0883	105.8494	
1938								
Mississippi	186.9561	520.8006	1.171.3228	206.5519	41.443.2050	1.323.7494	368.2050	
North Carolina	105.8856	219.7256	164.8556	81.4480	10.775.1200	247.5200	88.0644	
Oklahoma								
Tennessee (Knoxville)	98.3811	316.1.8516	1.3402	1.9480	159.0480	2.9254	165.4080	
Texas (Greenville)	65.0867	136.4578	38.1800	136.6933	6.938.0200	108.9422	2.5816	
New Mexico	67.1756	96.5200	49.0394	5.724.5000	99.9867	12.6978	118.4978	
1939								
	2.0820	4.6146	.6918	.8035	58.8613	1.4026	.8828	

## YIELD OF SEED COTTON, IN POUNDS, OF TABLE 6

Year and location	Mean squares <sup>1</sup>						Total Error (b)
	Rows	Columns	Skips	Error (a)	Positions	Positions × rows	
1936							
Louisiana	0.003697	0.078631	0.033058	0.009338	1.018165	0.016778	0.004796
1937							
Louisiana	.025159	.050946	.120713	.0158530	3.181503	.115026	.020806

<sup>1</sup> Degrees of freedom: 199 for total; 9 for rows, columns, skips, positions × rows, positions × columns; 1 for positions; and 72 for errors (a) and (b).

The following equation is suggested for single-row plots if skip distances are measured from the main stem of plants adjacent to the skip:

$$\text{Adjusted yield} = \text{actual yield} \times \frac{\text{total row length in feet}}{\text{total row length} - (\text{skip distance} - 3 \text{ ft.})}.$$

If distances are measured from the tips of branches of the plants adjacent to the skip, the equation suggested for single-row plots is as follows:

$$\text{Adjusted yield} = \text{actual yield} \times \frac{\text{total row length in feet}}{\text{total row length} - \text{skip distance}}.$$

#### DISCUSSION

In applying the method of skip correction indicated by these studies to extensive cotton variety tests, it has been found that the adjusted yields usually were reasonable in amount. In a relatively small number of cases the correction obtained by applying the formula appeared to be either too great or too small, and in such cases it was difficult to determine whether local soil variability or the formula should be suspected as responsible for the apparent discrepancy. The approximate equality in number of cases of suspected overcorrection and undercorrection indicates that the formula is without detectable bias and suggests that apparent discrepancies may be due largely to soil heterogeneity of the type that occasionally causes wide differences in yield among individual rows in multiple-row plots.

The need for an upward adjustment in yield of single-row plots containing skips and the evidence that compensation occurs laterally in adjacent rows raise the question whether a downward adjustment should be made in the yield of rows adjacent to those containing skips. Studies of average yields of outside rows for the nine locations in 1936 indicated a slightly higher yield for outside rows adjacent to longer skips than for those adjacent to shorter skips. In the analyses of variance for single locations the effect of skip length on yield of outside rows exceeded the 5-percent level of probability at three locations and was nonsignificant at the other six. Since the advantage gained by outside rows from different skip lengths did not reach high significance (odds 99:1) at any location and reached the level of interest, or low significance (odds 19:1), at only one-third of the places, the need for adjustment is by no means clear. These tests were based on an average of the two outside rows and thus reflect a cumulative advantage. It follows that, if tests were made on single outside rows, the expectation of finding significant advantages would be less. From all the evidence available it may be concluded that in the majority of cases gains in single rows adjacent to skips on one side are likely to be within experimental error. Under such circumstances it seems doubtful if a downward adjustment should be made in rows adjacent to those containing skips.

Average yields of seed cotton from whole plots, for each year and for the 3-year period 1936 to 1938 at the Delta Branch Station, Stoneville, Miss., were summarized by Neely.<sup>7</sup> Conclusions and recommen-

<sup>7</sup> NEELY, J. W. IRREGULAR STANDS MAY NOT DECREASE YIELDS OF COTTON. Miss. Farm Res. 4 (4): 1, 8. 1941.

dations coincide with those from the whole study. Since the experiments at this station were conducted on fertile Delta soil and others in the study were carried out at locations representing various levels of fertility and a wide range of climatic conditions, the agreement in conclusions from the various experiments indicates that the compensation for missing segments is similar under a wide range of soil and climatic conditions.

Yields of middle rows and whole plots for the three experiments conducted at Baton Rouge, La., have been summarized by Cotton.<sup>8</sup> The conclusions from these studies on the necessity for adjusting yields coincide substantially with those from the whole study. There is some evidence that, under conditions of heavy and well-distributed rainfall, corrections in single-row plots may not be necessary for skips less than 5 or 6 feet in length.

#### SUMMARY

Twenty-seven skip-correction tests, based on perfect-stand checks and skip distances of 2, 3, 4, 5, 6, 7, 8, 9, and 10 feet in the middle row of 3-row 25-foot plots, were conducted during the 4-year period from 1936 to 1939. At each location the experiments were arranged as 10 by 10 Latin squares. Yield data were obtained by individual rows, and boll counts or weights of seed cotton were taken on 3-foot segments in the middle row and on certain segments of outside rows.

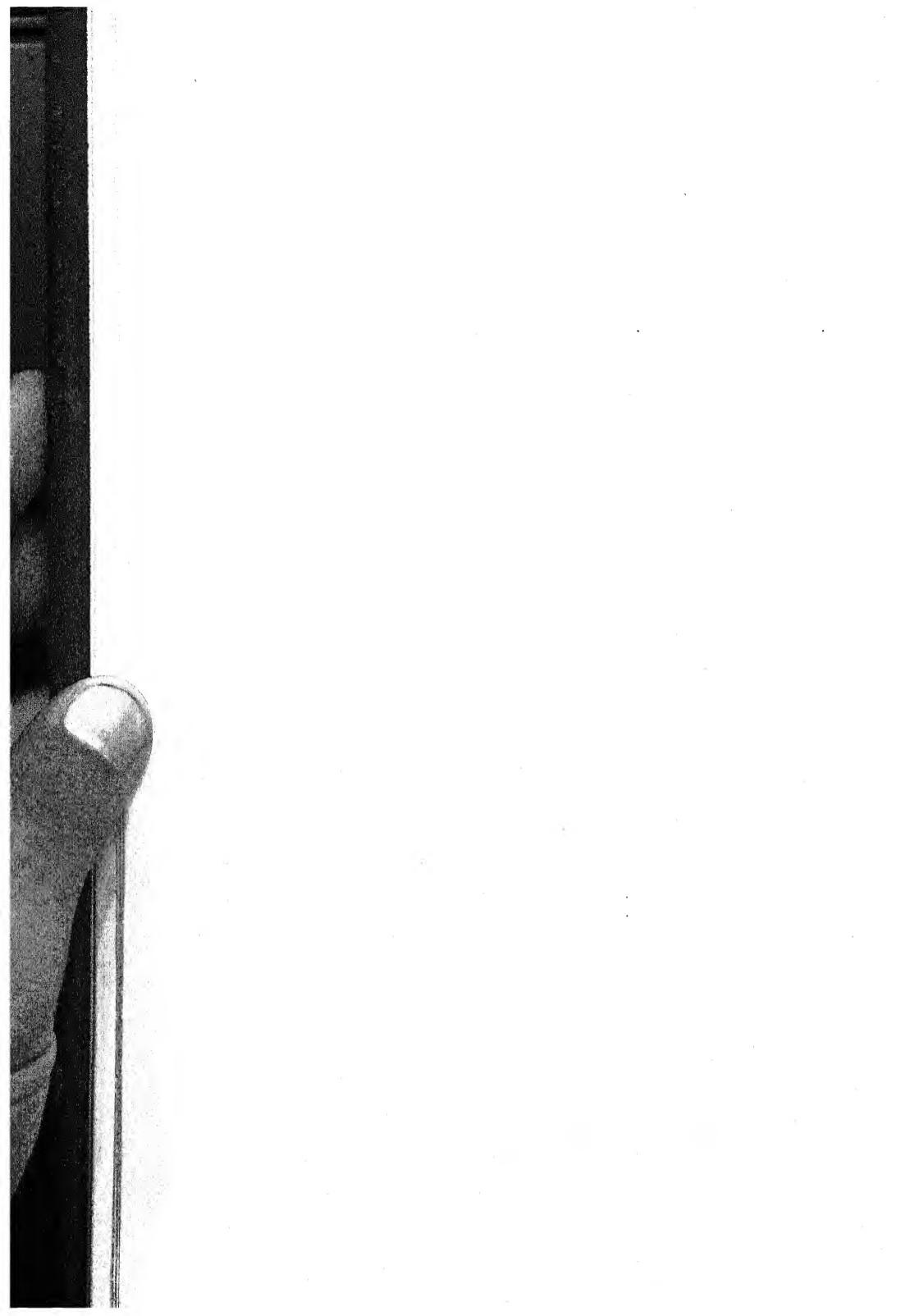
Yield studies were made both on the whole plots and on the middle rows in order to evaluate the effect of skips, or missing segments of row, on yield. The whole-plot analyses indicated a slight but non-significant reduction in yield with increasing skip increments.

Analyses of variance on yield of middle rows showed a significant reduction in yield due to skips in each of the 27 experiments, and this reduction was largely linear beyond a skip distance of 3 feet.

Analyses of variance were made on boll counts for the first and second 3-foot segments adjacent to the skip in the middle rows of each plot. The means and analyses of variance indicated that there was compensation by plants in the middle-row segments adjacent to the skip but that this compensation was largely independent of skip length, which suggests that the end plants may compensate for only a limited portion of the reduction in yield due to the skip.

From all the information available from these 27 tests 2 conclusions appear reasonable: (1) Skips occurring on interior rows of multiple-row plots are largely compensated for by increased production on end plants in the row containing the skip and by lateral compensation on the adjacent rows; (2) in single-row plots there is a definite reduction in yield due to skips, and this reduction appears to be substantially linear for distances greater than 3 feet. Consequently, for single-row plots a linear correction for skip distance minus 3 feet may be expected to give a satisfactory adjustment in computing yields if skip length is measured from stalk to stalk of plants adjacent to skips. If measurement is from nearest branches of plants adjacent to skips, a linear correction for actual skip distance is suggested.

<sup>8</sup> COTTON, J. R. SOME PRELIMINARY NOTES ON A SKIP CORRECTION STUDY IN LOUISIANA. Paper presented at meeting of the Association of Southern Agricultural Workers, New Orleans, La., 1939. [Unpublished.]



# PHOTOPERIODIC BEHAVIOR OF MARYLAND MAMMOTH TOBACCO UNDER LOCALIZATION TREATMENTS AND IN GRAFTS WITH CONNECTICUT BROADLEAF<sup>1</sup>

By H. A. ALLARD

*Senior physiologist, Division of Tobacco, Medicinal, and Special Crops, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture*

## INTRODUCTION

The Maryland Mammoth variety of tobacco (*Nicotiana tabacum* L.) is distinctive in having short-day requirements; that is, it normally flowers in response to naturally shortening days in northern latitudes. For this reason it is a very late flowering variety when grown commercially in the field in Maryland. This behavior is in marked contrast to that of all other commercial varieties of tobacco, which are day-neutral in their flowering behavior.

Various workers have concluded from their experimental results that the stimulus which initiates flowering originates in the leaves of the plants and not in the tissues of the growing point. This influence, the actual nature of which is unknown at present, is considered to affect other portions of suitably treated plants and to cause flowering in these even when they never have been subjected to a favorable photoperiod. The view generally held at present is that a flowering hormone or chemical substance capable of diffusing from cell to cell ultimately reaches remote tissues of a plant or graft, causing it to flower without the stimulus of a favorable photoperiod.

## LOCALIZATION TREATMENTS ON BRANCHES OF MARYLAND MAMMOTH TOBACCO

Localization treatments were designed to determine whether short-day conditions favorable to flowering localized upon the leaves of branches of Maryland Mammoth plants would induce flowering in other portions of the same plant subjected to lengths of day in excess of that critical for flowering. Seed of the Maryland Mammoth tobacco was sown January 21, 1943, and germinated January 27, and the plants were potted in thumb pots February 22. These were transferred to 4-inch pots April 14, to large pails June 7, and to DeWitt cans July 2. For the localization tests, three lightproof, ventilated cases of wood with the following inside dimensions were used: Height 38½; width 22½; and length 28½ inches. The east, west, and north sides were fitted with removable doors. During the tests a

<sup>1</sup> Received for publication December 14, 1944.

daylight period of 8 hours and 15 minutes was allowed that portion of the plant within the case, the doors being removed at 8 a. m. and replaced at 4:15 p. m. The three tests began July 23.

In the first test a vigorous young Maryland Mammoth plant 29 inches tall was so placed on the south side of the case that eight large leaves of one side of the plant were entirely within the case. The spaces between the leaves were then closed by heavy cardboard and black cloth so that, when the east, west, and north doors were closed from 4:15 p. m. to 8 a. m., these leaves were completely darkened, being exposed each day, as previously stated, to about 8 hours of light, a photoperiod conducive to flowering in the Maryland Mammoth variety.

The stem and leaves remaining outside experienced the full day. The dimensions, in inches, of the 8 darkened leaves at maturity were as follows: 16 by 11; 14 by 7½; 15½ by 8¼; 17 by 8; 17 by 8¼; 17½ by 9; 15½ by 7; and 17 by 7. By October 12 all these leaves had died. On the stem outside the case, subjected to the full seasonal length of day, flower buds did not appear until September 10. The first flowers opened September 16, 55 days after the test began, the plant at that time having a height of 90 inches. Fifty-eight leaves were counted to the first flowering branch of the inflorescence. This treated plant flowered no earlier than some of the control, untreated Maryland Mammoth plants. The control plants budded and flowered as follows. Plant 1: Buds, August 25; flowers, September 10 at 90 inches. Plant 2: Buds, August 25; flowers, September 3 at 86 inches. Plant 3: Buds, August 30; flowers, September 10 at 90 inches. Plant 4: Buds, August 25; flowers, September 1 at 85 inches. These plants produced approximately 65 to 70 leaves, counting all leaf scars and leaves to the first flowering branch. The 3 largest leaves averaged about 20 by 7½ inches.

In the second test, which also began July 23, a Maryland Mammoth plant which had produced two vigorous lateral branches as the result of cutting back the main stem about 7 inches from the ground was used. The plant and container were kept outside on the south side of the case. One branch 8 inches long was placed within, and the stem was defoliated and wrapped with black cloth where it passed through the opening in the case so that no light could enter there. When the test began, the outside branch was 16 inches long as measured from the ground. The inside branch was afforded about 8 hours of light as in the first test, since the same schedule of opening and closing was observed. The outside branch was kept defoliated from the beginning of the test, only the youngest, topmost leaves below the growing point being left as the defoliation was continued.

Defoliation appears to have produced no effect, unless it was an actual slight delay in flowering. Buds appeared on the inside branch August 30, 38 days after the test began, and the first open flowers September 29, 68 days from the beginning of the test, when the branch had reached a height of 37 inches. At this time the three largest basal leaves measured 23 by 8, 21 by 7½, and 23 by 9½ inches, respectively. Thirty-four leaves had developed to the first flowering branch.

The outside defoliated branch experiencing full day showed buds September 29, at the end of 68 days, just 30 days later than the inside branch. The first open blossoms appeared October 9, 78 days after the test began, at a height of 45 inches. Seventy-eight leaves had been removed from the outside stem. This stem, remaining slender and scarcely longer than the inside flowering stem, bore only a small inflorescence. The stem was considerably smaller than those of the control plants, which were afforded the full day and had not been defoliated. However, both flowering dates were later than those for the four controls discussed in connection with test 1.

In the third test, also begun on July 23, a Maryland Mammoth plant was cut back and arranged as in test 2 so that one branch remained within the case. This branch was afforded about 8 hours of daylight, while the one without experienced the full day. Neither branch was defoliated.

On November 12, 112 days after the test began, the inside branch, subjected to the short photoperiod, had not budded, was only 26 inches long, and had produced only 24 leaves, counting all to the terminal bud. The outside branch, experiencing the full day, showed buds September 10 and the first open flowers September 22, 61 days after the test began, at a height of 74 inches. However, both flowering dates are later than those for the 4 controls discussed in connection with test 1. On November 12 the outside branch had attained a height of 80 inches and had produced 54 leaves. The 3 largest outside leaves measured 20½ by 6½, 21 by 7, and 20 by 6½ inches, respectively. The leaves of the inside branch were noticeably smaller and narrower, the largest leaf being 18 by 5½ inches.

Since flowering in the Maryland Mammoth variety is favored by short days, the failure of the inside branch to flower under the conditions of this localization test appears somewhat anomalous. It is possible that some lack of physiological balance, brought about by the two competing shoots under these conditions, delayed flowering in the shoot experiencing the short photoperiod. It is also possible that these tests were begun too late in the season and therefore too near the critical length of day to get striking differences in time of flowering.

The experiments with Maryland Mammoth, in the same lightproof cases and with the same methods of treating and darkening the plants, were repeated in 1944, the tests beginning May 29. In one test the outside receptor branch was defoliated and wrapped with lightproof black cloth from June 5 until July 18, 43 days. The inside branch receiving about 8 hours of light daily flowered July 14, 46 days after the tests began. The defoliated and wrapped outside branch had shown no buds September 1, 95 days after the tests began.

In a second test, also begun May 29, the outside receptor branch was defoliated even to the removal of all the auricles from July 18 to September 1, 45 days. The inside darkened branch showed buds about August 29. At this time the outside defoliated receptor branch had not budded. These tests possibly indicate that the 1943 experiments were begun too late in the season to secure pronounced differences in flowering. Even in these later tests, there was no

indication that the receptor branch experiencing a length of day too long to induce flowering received any impulse conducive to earlier flowering even when kept defoliated or when defoliated and also wrapped with lightproof black cloth.

#### MARYLAND MAMMOTH IN GRAFTS WITH CONNECTICUT BROADLEAF TOBACCO

The publication of the early work of Garner and Allard (7),<sup>2</sup> showing the results of localizing different length-of-day treatments upon the branches of a plant, stimulated a vast amount of similar experimental work on various plants. It would appear that the photoperiodic stimulus tends to remain localized in its effects and confined to the treated branch as long as the rest of the plant experiences a length of day unfavorable to flowering and is not given additional treatment. If, however, the leaves of the branch which does not flower are removed or rendered more or less functionless by darkness, the flowering stimulus operates as well upon this branch, which has been termed the "receptor," and the initiation of flowering results. The experiments presented in this section were designed to determine whether this behavior also holds true in the case of the short-day Maryland Mammoth tobacco when grafted with the day-neutral Connecticut Broadleaf variety. Both plants were used as stock and scion. One lot was grown in beds in a rich soil, where the plants were afforded a much greater amount of space and soil for root development than pails or DeWitt cans provide. In other tests 14-quart pails or DeWitt cans of 32-quart capacity were used. Presumably owing to their limited soil capacity, these contained produced smaller and somewhat earlier flowering plants, as can be seen from a comparison of the data of tables 1 and 2.

For the plants grown in the west bed, seed of the Maryland Mammoth variety (1935 harvest) was sown September 7, 1942. This germinated September 12. The plants were transplanted to thumb pots October 18 and to pails November 17 and were cut back to a height of 4 to 5 inches from the soil in the spring. The grafts were made on the dates indicated in tables 1 and 2. Some of the plants were transferred to the beds June 4; the data on the behavior of these are shown in table 1. Some of the plants remained in pails or were transferred to DeWitt cans; the data for the behavior of these are shown in table 2. The Connecticut Broadleaf plants used for all the grafts were obtained from seed sown January 21, 1943. This germinated January 27. The plants were transferred to thumb pots February 22.

The data of table 1 are derived from plants grown in 2 beds, 16 plants in each bed. Plants 1 to 16, Maryland Mammoth variety, were used as stocks upon which 1 or 2 scions of Connecticut Broadleaf were grafted. Plants 17 to 26, Connecticut Broadleaf variety, were used as the stocks grafted with 1 or 2 scions of Maryland Mammoth. Plants 27 to 32, Maryland Mammoth variety, were not treated in any way and were used as controls. The Maryland Mammoth

<sup>2</sup> Italic numbers in parentheses refer to Literature Cited, p. 30.

branches of plants 2 to 8 were kept defoliated from June 18. The leaves were kept removed to the tip, leaving only the small, very immature bud leaves. The defoliated portion of the stalk of plant 3 was also kept covered with light-impervious black cloth.

Table 1 shows that not one of plants 2 to 8, the Maryland Mammoth branches of which were defoliated or defoliated and wrapped with black cloth, had shown buds until after November 20, 221 days after the grafts were made, although defoliation of these plants had been maintained for 155 days. Since the Connecticut Broadleaf scions flowered through a range of 77 to 116 days after the grafts were made, there was a period ranging from 105 to 144 days after the flowering of the scions during which any active flower-forming material originating in the plants could have operated as a flowering stimulus in the Maryland Mammoth branches.

Plants 9 to 16 represented grafts of Connecticut Broadleaf scions upon Maryland Mammoth stocks without any additional treatments given to either stock or scion. Since the Connecticut Broadleaf scions in this series flowered within a range of 77 to 108 days after the grafts were made and the Maryland Mammoth branches had not budded until after November 20, 221 days from the date of grafting, it is evident that there was an interim of 113 to 144 days when flower-forming material could have been available to these Maryland Mammoth branches. This would depend upon the assumption that it became available only at the time of blossoming of the Connecticut Broadleaf scions.

The behavior of these defoliated and nondefoliated groups of plants would indicate that the defoliation treatments of the Maryland Mammoth branches had failed to hasten the formation of buds or flowers under the conditions of these experiments.

Plants 17 to 26 represented grafts in which the Connecticut Broadleaf served as stocks and the Maryland Mammoth as scions. In this group, excluding plants 18, 19, and 21, the Maryland Mammoth scions and the branches of the Connecticut Broadleaf stock were allowed to develop with no further treatment after the grafts were made. The scions of two plants in this group, 18 and 19, budded or flowered in advance of the rest; plant 18 showed buds October 20, 190 days from the date of grafting, but subsequent flowering seemed to be delayed. Other plants in this group had not budded in October but had budded or were in flower in early December, more than 231 days after the date of grafting. Plant 19 flowered in 183 days.

Plants 27 to 32, variety Maryland Mammoth without grafts or treatment of any kind, served as controls. Only one, plant 28, had flowered by October 12, 182 days from the time of the grafting with Connecticut Broadleaf plants. The other plants did not bud until after November 20, at least 221 days after grafting.

Table 2 presents data for 38 plants grown either in pails or in the much larger DeWitt cans. Plants 1 to 21 were Maryland Mammoth upon which 1 or 2 scions of Connecticut Broadleaf had been grafted; the scions failed to unite on plants 1 to 5 and 10. Plant 6 failed to develop a branch from the Maryland Mammoth stock. Scions of Connecticut Broadleaf grew successfully on plants 11 to 18.

TABLE 1.—Responses of late-flowering, short-day Maryland Mammoth and of early-flowering, day-neutral Connecticut Broadleaf tobaccos in grafts and of ungrafted Maryland Mammoth grown in beds

[Grafts made April 13, 1943]

Scion, stock, and plant No.	Scions used	Treatment of stock	Treatment of scion	Response of Connecticut Broadleaf				Response of Maryland Mammoth 1				
				Appearance of buds		Appearance of flowers		Appearance of buds		Appearance of flowers		
				Date	Scion or stock re-spond- ing	Date	Period from grafting to flowering	Date	Period from grafting to flowering	Date	Period from flowering of Connecticut Broadleaf to that of Maryland Mam- moth	
Connecticut Broadleaf on Maryland Mammoth:	Number: 1	None-----	1 kept defoliated from June 6. 1 branch kept de- foliated from June 18, leaving only small leaves at tip.	{ 1 2	{ 1 2	June 4 July 7	Days Inches 32 85	{ 30 } Late ber.	{ Nov. 30 } do	231 do	{ 179 146	
	2	None-----	None-----	July 24	2	{ July 30 [Aug. 7	108 80	{ 64 80	{ do } do	231 do	{ 123 115	
	3	-----	do-----	June 25	1	July 6	84	50	do-----	231 do	{ 147 250	
	4	-----	Branch kept de- foliated to tip from June 18. Defoliated portion covered with lightproof black cloth.	do-----	do-----	1 July 7	85	59	do-----	231 do	{ 255 146	
	5	-----	Branch kept de- foliated to tip from June 18. This remained slender.	do-----	do-----	June 24	1 July 28	106	75	do-----	231 do	{ 266 125

See footnotes at end of table.

TABLE 1.—Responses of late-flowering, short-day Maryland Mammoth and of early-flowering, day-neutral Connecticut Broadleaf tobaccos in grafts and of ungrafted Maryland Mammoth grown in beds—Continued

Crests made April 13 1943

Section, stock, and plant No.	Scions used	Treatment of stock used	Response of Connecticut Broadleaf				Response of Maryland Mammoth 1				
			Appearance of buds		Appearance of flowers		Date of appear- ance of buds		Date		Appearance of flowers
Treatment of scion	Date	Scion or stock or re- spond- ing	Date	Period from grafting to flower- ing	Height	Period from grafting to flower- ing	Height	Days	Inches	Days	Inches
Maryland Mam- moth on Con- necticut Broad- leaf—Con. 19-----	1	Branch cut back June 18 and again July 21 to promote growth of the dwarfed Mary- land Mammoth scion.	July 23	Branch not treated.	Aug. 1	Days 112	Inches 69	Oct. 7-----	Oct. 13	Days 183	Inches 89
20-----	1	Cut back branch July 2, as the Maryland Mam- moth scion re- mained very small with leaves not more than 2-2½ inches long.	July 12	do	July 24	102	74	Late November ----do----	---	231	1160 336
21-----	1	Branch not treated.	July 24	1	Aug. 11	120	82	----do----	---	231	1129 111
22-----	1	Branch not treated.	July 7	1	July 17	95	73	----do----	---	231	1126-156 136
23-----	1	do-----	July 24	1	July 29	107	88	----do----	---	231	174
24-----	1	do-----	July 11	1	July 17	95	80	----do----	---	231	1170
25-----	1	do-----	July 24	1	Aug. 5	114	79	----do----	---	231	1120
26-----	2	Branches not treated.	June 29	2	July 7	85	59	----do----	---	231	136-100 146

grafted and untreated Maryland Maple (controls); 27.	Abical branch- ing memory to flower- ing in late November.	(4)	1172
28.			
29.			
30.			
31.			
32.			

- 1 All contracts for Mammoth branches were fulfilled or in process when the experiment was discontinued in early December, unless otherwise indicated.
- 2 The contracts for Maryland Mammoth branches had not been filled by Oct. 12. The defoliated Maryland Mammoth branches showed no new growth.

gns of buds when the experiment was discontinued in early December.  
3 No buds evident when the experiments were discontinued in early December.

<sup>4</sup> Time of flowering computed from April 13, when Maryland plants of

er 30 unless some other date given.

52 branches developed.

2. *Wattles* *versicolor*.

TABLE 2.—Responses of late-flowering, short-day Maryland Mammoth and of early-flowering, day-neutral Connecticut Broadleaf tobaccos in grafts and of ungrafted Maryland Mammoth grown in pairs or DeWitt cans, 1948

Plant No.	Date of grafting	Scions used	Condition or treatment of scion or stock	Response of Connecticut Broadleaf				Response of Maryland Mammoth			
				Appearance of flowers		Date of appearance of buds	Length of scion from grafting to flowering	Period from grafting to flowering	Date of appearance of buds	Length of scion from grafting to flowering	Period from grafting to flowering
				Date	Days						
1.	Apr. 23	Number 1	Scion did not unite; 2 branches of stock developed.			Sept. 10	Sept. 28	{ 158	Inches 61	Days 61	
2.	do	1	Scion did not unite; 1 branch of stock developed.			Sept. 22	Sept. 29	{ 152	62		
3.	do	1	Scion did not unite; 2 branches of stock developed.			Sept. 10	Sept. 29	{ 159	36		
4.	do	1	Scion did not unite; 1 branch of stock developed.			Sept. 6	Sept. 13	{ 143	85		
5.	do	1	Scion did not unite; 2 branches of stock developed.			Sept. 10	Sept. 22	{ 152	68		
6.	do	2	Scions united; stock failed to develop branches.	June 20	July 8	76	45		152	65	
7.	do	1	Scion united; 1 branch of stock developed.	July 8	July 20	88	43				
8.	do	1	Union of scion weak; stock developed 1 branch.	July 12	do	88	40				
9.	do	1	Scion united; stock failed to develop 1 branch.	Sept. 10	Sept. 29	159	40				
10.	do	1	Scion did not unite; stock developed 2 branches.	June 30	July 17	85	53				
11.	do	1	Scion united; stock developed 1 branch.	Sept. 10	Sept. 22	152	55				
12.	do	1	do	July 10	do	88	45				
13.	do	1	do	July 31	do	90	40				
14.	do	1	(Scion grafted April 13 failed to unite; second scion cut back from 30 to 18 inches June 4; stock developed 2 branches.	June 29	July 8	75	54				
15.	Apr. 13 (Apr. 24)	2	Scion united; stock developed 1 branch.	June 15	June 15	53	50				
16.	Apr. 23	1	do	July 8	114	53	50				
17.	Mar. 16	1	do	July 10	126	44	44				
18.	do	1	Scion united; stock kept defoliated to dip from July 24.	July 15	July 31	99	44				
19.	Apr. 23			July 15	July 31	99	44				
						36	Nov. 9	197	76	71	122
						36	Nov. 9	197	76	71	122
						36	Nov. 9	197	76	71	122

20	do.	2	Scions failed to unite; 1 branch of stock kept defoliated to tip from June 18.											
21	May 11	1	Scion united; stock defoliated to tip from June 18.											
22 <sup>5</sup>														
23														
24														
25														
26														
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30														
31														
32														
33 <sup>6</sup>														
34														
35														
36														
37														
38														

<sup>1</sup> On Oct. 12 plant height 65 inches; no buds evident.<sup>2</sup> Computed from April 24, date of grafting of scion that united.<sup>3</sup> Defoliated portion 34 inches.<sup>4</sup> None by Nov. 13, when plants were 22½ inches tall.<sup>5</sup> Plants 22 to 32, inclusive, in 14-quart cans as controls. Flowering period computed from April 23.<sup>6</sup> Plants 33 to 38 inclusive, in 32-quart DeWitt cans as controls. Flowering period computed from April 23.

In this group of 8 plants, the Connecticut Broadleaf scions began to flower through a period extending from 53 (No. 16, flowering June 15) to 152 days (No. 11, flowering September 22) from the date of grafting, whereas the branches from the Maryland Mammoth stocks of plants 11 to 18 flowered through a period extending from 151 to 197 days from the date of grafting. In these grafts the Connecticut Broadleaf scions had flowered from 7 to 115 days before the branches from the Maryland Mammoth used as the stock.

Since plants 1, 3, 4, 5, and 10 on which the Connecticut Broadleaf scions had failed to unite flowered through a period extending from 143 to 159 days, it would appear that the floriferous Connecticut Broadleaf scions on plants 11 to 18 had not hastened flowering of the Maryland Mammoth branches.

Two plants, 19 and 21, upon which scions of Connecticut Broadleaf were growing, received additional treatment. The Maryland Mammoth branches on plant 19 were kept defoliated from July 24 and those on plant 21 from June 18. The Maryland Mammoth branch of 19 showed buds in 200 days from the date of grafting but did not flower until the last week in November, about 221 days from the date of grafting. On the Maryland Mammoth branch of plant 21, which had been grafted with Connecticut Broadleaf May 11, buds had not appeared as late as November 13, 186 days from the date of grafting. The period of defoliation of plant 19 amounted to 119 days to November 20 and of plant 21 to 155 days. It is possible that defoliation actually delayed flowering in these instances, since control plants 22 to 38, which were grown either in pails or in the larger DeWitt cans and had not been grafted or given other treatments, flowered somewhat earlier, with a range of 63 to 169 days. Plant 20 was also grafted with a scion of Connecticut Broadleaf April 23, but this did not unite. One Maryland Mammoth branch on this plant was defoliated from June 18 and not until November 13, 204 days after the graft was made, or after 148 days of defoliation, did it flower. None of the controls flowered much later than 152 to 169 days after April 23, whereas the three defoliated plants (19, 20, and 21) flowered at least 200 days after that date; plant 19 was only in bud 200 days after April 23 and plant 20 flowered 204 days after that date.

A comparison of the data of tables 1 and 2 with respect to time of budding or flowering shows that there was a noticeable delay in the beds. If the time of flowering of the untreated controls in the two groups is compared, it will be seen that in most instances actual flowering in the containers occurred within about 160 days. The controls in the beds, on the other hand, had shown no buds even at the end of a period of 182 days and flowering was delayed to about 231 days in most cases.

Three plants, 28, 29, and 30, in the group of controls presented in table 2, flowered within a period of 63 to 67 days, whereas the majority of the other plants in this lot required 152 to 160 days. Abnormally early flowering in these instances is probably explainable by the fact that when cut back in the spring, after producing seed all winter, these three plants for some reason still retained enough of a residual flowering stimulus to cause seemingly premature flowering independ-

ently of the summer length of day, which was then far above the critical length and distinctly unfavorable for flowering in this short-day variety.

Data of tables 1 and 2 indicate that where grafts had been made between the short-day Maryland Mammoth variety and the day-neutral Connecticut Broadleaf, there was no hastening of flowering in the Maryland Mammoth branches as a result of transfer of some flower-producing material or stimulus from the early-flowering Connecticut Broadleaf. This negative behavior was shown whether the Maryland Mammoth was used as stock or scion. Furthermore, there was no indication that defoliation of the Maryland Mammoth branches facilitated any movement of an active flower-inducing stimulus into such branches under the conditions of these experiments. In fact, there appears to be some indication that defoliation delayed rather than hastened flowering.

#### DISCUSSION AND CONCLUSIONS

Workers who have critically studied the length-of-day responses of plants are generally agreed that the flowering stimulus originates in the leaves of plants subjected to a suitable length of day favorable to flowering. Among these are Cajlachjan (4), Cajlachjan and Aleksandrovskaia (5), Fabian (6), Knott (10), Kuijper and Wiersum (11), Lubimenko and Bouslova (12), Moskov (15), Zimmerman and Hitchcock (18), Melchers (19), Hamner and Bonner (8), and Hamner and Long (9). Furthermore, it has been reported by Borthwick and Parker (2) for soybeans, by Hamner and Bonner (8) for cocklebur, and by Withrow et al. (17) for spinach that this stimulus under favorable conditions of treatment can induce flowering in the branches of a plant that has never experienced a favorable length of day. Some workers have assumed that this stimulus depends upon a diffusible flower-inducing substance that has formed in response to a particular photoperiod.

The data obtained in the localization and defoliation tests with the Maryland Mammoth plants are in agreement with those obtained when an early-flowering variety, Connecticut Broadleaf, was grafted with the short-day, late-flowering Maryland Mammoth variety (tables 1 and 2). In no instance was it indicated that any flowering stimulus was imparted to Maryland Mammoth branches experiencing a length of day above that critical for flowering, either from the Connecticut Broadleaf branches in grafts or from branches that were given short-day conditions favorable to flowering.

In the light of the differences in behavior that have been reported by various workers with other species, both short-day and long-day types, the results reported in the present paper do not appear unusual.

Borthwick and Parker (2) reported that when one branch of a two-branched Biloxi soybean plant was subjected to short photoperiods favorable to flowering at the same time that another was subjected to long photoperiods above the critical for flowering, the initiation of flower buds did not extend to the receptor branch until this had been defoliated. The presence of leaves upon the receptors appeared to

keep the flowering impulse localized upon the donors. Borthwick and Parker also showed that the flowering impulse arises in the leaves.

Hamner and Bonner (8) showed that cocklebur (*Xanthium pensylvanicum*) departs somewhat from this behavior. If one branch of a two-branched plant of cocklebur is exposed to a photoperiod favorable to flowering, the receptor branch experiencing long photoperiods above the critical for flowering will also flower even though it has not been defoliated. Hamner and Bonner showed that the stimulus responsible for flowering originates in the leaves of the cocklebur and that one leaf given short photoperiods is sufficient to cause the initiation of flower buds in other portions of the plant subjected to photoperiods too long to induce flowering. Similar results reported by Borthwick and Parker (3), working with Biloxi soybeans, also showed that individual leaves given the proper photoperiod can induce floral initiation.

Withrow et al. (17), working with Nobel spinach, a plant flowering in response to long days, reported that when the leaves of a plant received long photoperiods favorable to flowering while the rest of the plant experienced short photoperiods unfavorable to flowering flower buds were initiated in the long-day portion. One leaf subjected to long photoperiods was not enough to induce flower primordia to form in a normal intact plant exposed to short photoperiods. However, if the rest of the plant was defoliated, one leaf was sufficient, showing that in the case of spinach the leaves in an unfavorable photoperiod exercised an inhibiting effect.

Moskov (16) grafted scions of *Nicotiana rustica* and *N. tabacum* var. Samsun on Maryland Mammoth stocks subjected to continuous light unfavorable to flowering. In the case of Samsun two methods of grafting, the cleft graft and the splice graft, were used; in the latter, scions were fitted and bound to branches of the stock on a single slanting cut. Moskov stated that the early-flowering scions, both *N. rustica* and Samsun, hastened flowering in side branches of the Maryland Mammoth but that Samsun had this effect only when the splice graft was used.

Melchers (14) grafted annual forms of *Hyoscyamus niger* L. with biennial forms and asserted that there was a change from the biennial vegetative habit to the annual flowering habit in the growing points. He stated that flowering scions of *H. albus* when grafted with the biennial *H. niger* induced the growing point of the latter to become flowering in its expression and that scions of the short-day Maryland Mammoth plant under short-day conditions also caused flower formation on stocks of the biennial *Hyoscyamus*. He failed to change the vegetative development of the growing point of this biennial stock when he used scions of tomato, however, but this he thought might have been due to a poor union of the graft.

The evidence presented under the conditions of the writer's experiments is somewhat at variance with some of these results. It is obvious that the uniformity of behavior shown in these tests with tobacco is not an accidental one. It is significant that *Xanthium* (8) did not behave in the same manner as Biloxi soybean (2), since the receptor in the case of *Xanthium* flowered without defoliation. The

Maryland Mammoth tobacco in the tests presented did not flower whether or not the receptor was defoliated.

When grafts are made between types of tobacco as different in their flowering behavior as the day-neutral Connecticut Broadleaf and the short-day Maryland Mammoth, the resultant combination becomes somewhat unbalanced or unstabilized. Often it is very hard to maintain an equilibrium of growth in the branches of scion and stock and so prevent one or more branches from becoming completely dominant and perhaps severely disturbing growth conditions in other branches. It would appear that the Maryland Mammoth branch under some conditions may become so vigorous and dominant as to direct to itself nearly all growth material formed by the plant and thus leave the Connecticut Broadleaf branch impoverished and stunted. It can be readily theorized that this may profoundly affect the flowering behavior of either branch and, consequently, hasten or delay flowering in one or another member if an actual flower-forming, transportable hormone is involved.

It would appear that results of work up to the present time indicate considerable variability in the behavior of different plants with respect to the effects of localization of photoperiods favorable to flowering and the transmission of the effects to other parts of a plant experiencing unfavorable photoperiods. While it is generally held that the stimulus for flowering originates in the leaf, there is no good reason why this should necessarily be so. The leaf itself is a complex assimilative organ, and in the case of tobacco it is sessile in its attachment, with large auricles extending around the stem or more or less decurrent upon it.

The petiole of the leaf, the leaf blade, and the large auricles contain an abundance of green pigment. The main stem and branches of the plant are also green and would appear to be capable of photosynthetic assimilation. It is for this reason that some of the plants (for example, plant 3, table 1) were not only defoliated but wrapped with light-impervious black cloth up to the apex of the branch. This treatment, however, as previously stated, did not appear to hasten flowering in the defoliated Maryland Mammoth branch.

Cycles of various alternations of light and darkness are of direct import in these studies. Hamner and Bonner (8), as a result of their studies of *Xanthium*, concluded that the dark period is more responsible than the light one for flowering in response to favorable photoperiods. They concluded further that, in any cycle of alternations of light and darkness, a dark period in excess of  $8\frac{1}{2}$  hours should cause flowering and, conversely, that any dark period shorter than  $8\frac{1}{2}$  hours should prevent flowering. While this may be true for the behavior of *Xanthium*, it is not true, as Allard and Garner (1) have shown, for some other plants.

Allard and Garner (1), working with Peking soybeans and other plants, showed that not only the ratio but also the absolute cycle within which the ratio is operative profoundly affected growth and reproduction. No conclusions could be drawn relative to a particular length of either the light or the dark period. Here again the behavior of *Xanthium* does not appear to be entirely comparable with that of the

Peking soybean or other plants. *Xanthium*, however, seems to be a typical example of a short-day plant with a very narrow critical photoperiod for flowering, since a difference of 15 minutes, according to Hamner and Bonner (8), may determine whether flowering will occur.

### SUMMARY

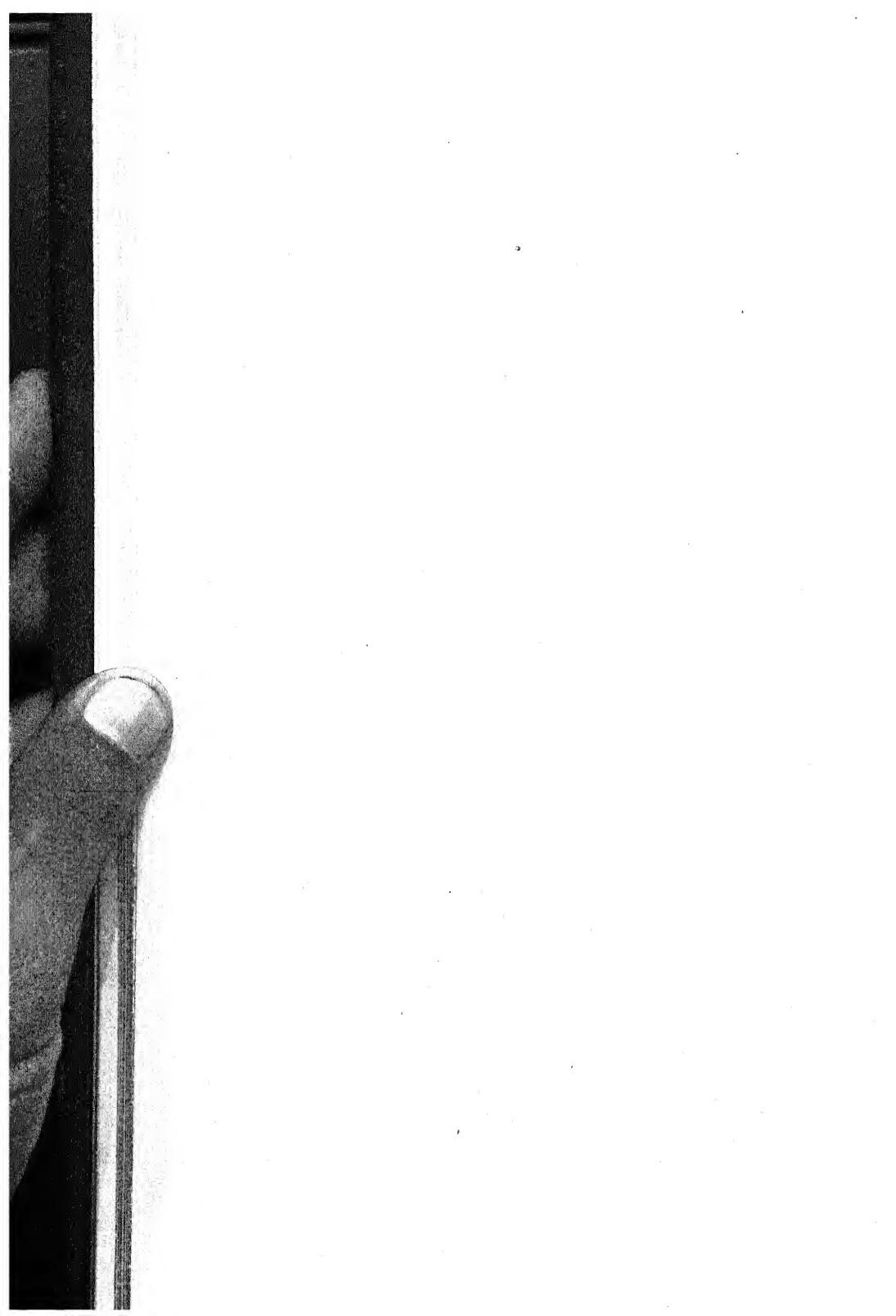
In localization tests on plants of Maryland Mammoth tobacco, lightproof cases were used to darken the leaves or branches, subjecting these to short photoperiods favorable to flowering. In one test the outside branch was kept defoliated. There was no indication of hastened flowering in any of these tests. It is possible that the tests were begun too late in the season for positive results, as indicated by a test during a second year.

Many grafts were made between the short-day Maryland Mammoth variety of tobacco and the day-neutral Connecticut Broadleaf variety, both being used as either stock or scion. Some of the Maryland Mammoth branches were kept defoliated for many weeks and one was both defoliated and wrapped with light-impermeable black cloth to determine whether such branches would flower earlier than untreated branches in similar grafts. There was no indication of transmission of a flowering stimulus from the flowering Connecticut Broadleaf stems (donors) to the defoliated or darkened Maryland Mammoth stems (receptors). In most instances there appeared to be delayed flowering in these treated stems.

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## SEASONAL CHANGES IN TOTAL AND SOLUBLE OXALATES IN LEAF BLADES AND PETIOLES OF RHUBARB<sup>1</sup>

By ALFRED T. MYERS<sup>2</sup>

Associate biochemist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture

### PURPOSE OF THE STUDY

The edibility of the leafstalks and the nonedibility of the leaf blades of rhubarb (*Rheum* spp.) are related in general to their content of soluble oxalic acid. The analyses reported in the literature, however, were not made on leaves of known ages. To help establish the best time to harvest rhubarb for market and the range of oxalic acid content, leaves of known age from plants growing at the Arlington Experiment Farm, Rosslyn, Va., were analyzed in 1932 and 1935. The data obtained are also of general biochemical and physiological interest. In the course of the study it was found necessary to evaluate, modify, and improve the method used by Bau (3, 4)<sup>3</sup> for the determination of oxalic acid in plant tissues.

### SOME EARLIER STUDIES

In 1886 Berthelot and André (5) found that in young plants of *Rumex acetosa* the oxalic acid content of the leaf blade exceeded that of the petioles, or leafstalks, and larger veins by a 12:10 ratio. In old plants the oxalic acid content of the leaf blade was lower than that of petioles and stems with a ratio of 4 to 5. In the leaf blade the acid was predominantly soluble in young and old plants, while in the petioles it was mainly soluble in the young ones and chiefly insoluble in the old ones.

In 1917 Steinmann (16) determined the titratable acidity of the petiole and leaf blade of rhubarb at various stages of maturity. He studied the effect of light and darkness on acid content and found an increase in acidity during a day's exposure to sunlight and a progressive decrease in leaves kept in darkness. There was a descending gradient of titratable acidity from leaf mesophyll to veins to petiole.

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<sup>3</sup> Italic numbers in parentheses refer to Literature Cited, p. 46.

Steinmann found evidence that the acid, like carbohydrates, was manufactured in the leaf tissue and that translocation downward followed; there was an increase in acid from the apex to the base of the leaf blade and from the veins to the petiole.

In 1917 Van Itallie and Lemkes (?) apparently found that rhubarb leaves contained from 0.30 to 1.11 percent of anhydrous oxalic acid while the petioles contained 0.44 to 0.99 percent. The toxic dose for man had been previously reported as 2 to 5 gm. of oxalic acid, but these writers believed it to be much lower. They noted one fatal case of poisoning from eating the green leaves.

In 1920 Angerhausen (2), using Bau's method of analysis (4), found that oxalic acid did not occur in rhubarb as oxalic acid but as soluble potassium acid oxalate to which the poisonous properties were due. He stated that potassium acid oxalate reacts with the blood and nerves and robs the latter of calcium. He found that the stalks contained 0.25 percent of soluble oxalate and the leaves 0.50 percent. He reported also that the oxalates in the petiole were almost if not wholly calcium oxalate.

Maue (10) in 1920 studied the constituents of rhubarb leaves in order to explain the fatal poisoning and sickness resulting when they were eaten as food. He and five other men ate cooked rhubarb leaves without the slightest ill effects. His analysis of the leaves showed 0.368 percent of soluble oxalate on the green-weight basis. After a physiological experiment he decided that the gastric juice of the stomach would not render the insoluble oxalates soluble. Maue believed that the illness and death sometimes resulting from the consumption of rhubarb leaves was possibly due to a saponin.

Later Ruhland and Wetzel (14, 15) found that the youngest petioles contain mostly succinic and malic acids and scarcely a trace of oxalic acid. As the age of the tissue increased there was a sharp decrease in the first two acids and a rapid increase in oxalic acid. These writers believed the high acid content to be a result of deamination of proteins rather than a partial oxidation of carbohydrates. They gave no description of their methods of identification or determination.

Culpepper and Caldwell (6) in 1932 determined the seasonal changes in titratable acidity of rhubarb leaves and petioles and found that the maximum total acid content is accompanied by most rapid cell division and increase in size in the young leaf as is the case with young fruits. The acid content of mesophyll tissue was consistently lower than that of the petiole throughout the season. The titratable acidity was greatest at the time of full photosynthetic efficiency.

In 1937 Pucher, Clark, and Vickery (11, 12) investigated the organic acid composition of rhubarb. They found that the leaf contained *l*-malic, oxalic, and citric acids together with acids of unknown nature. According to these workers (12, p. 616), "the composition differs in different parts of the leaf and is profoundly influenced by the age of the leaf and by the season in which it has developed." Their data showed the predominating acid of the petiole to be *l*-malic with oxalic in second place; citric and unknown acids were present in small amounts. In the younger leaf blades they found the unknown acids to predominate with oxalic acid next in amount. In leaf blades developed late in the season oxalic acid predominated over the un-

known acids and *l*-malic and citric acids were present in small amounts. Their data on concentration showed that oxalic, citric, and unknown acids occurred in a gradient that increased from petiole to veins to blade. They were interested more in shifts in composition of the mixtures of the different organic acids in young leaves as compared with old ones than they were in any detailed change in a specific organic acid.

Allsopp (1) in 1937 made an extensive study of seasonal changes in the organic acids of rhubarb. He estimated the seasonal changes in actual content of acid in the whole plant by referring the acid content in milligram equivalents to the original piece of resting rhizome from which the plant developed. He also gave the milligram equivalents on a fresh-weight basis (or percentage  $\times$  1,000). He analyzed rhizome and shoots (leaves) sampled once a month for 12 months and found marked changes in concentration of malic, citric, oxalic, and unknown acids. His data showed no increase in the amount of any of these acids on sprouting of the rhizome. All of them increased continuously in the leaves during the season of active photosynthesis, suggesting an origin due to photosynthesis or an indirect one from carbohydrates. Also, acids increased in the rhizome during the summer, indicating translocation from the leaves throughout the period of acid formation. Allsopp showed an initial fall in oxalic acid with the growth of the young leaves, after which this acid increased continuously. He reasoned that the marked changes in acidity, particularly the decreased content of all the rhizome acids during the winter, indicated that acids play an active role in metabolism and are not merely passive end products as some workers have suggested.

## MATERIAL AND METHODS .

### MATERIAL

The material on which these studies were based consisted of a 5-year-old planting of the Ruby variety of rhubarb grown at the Arlington Experiment Farm, Rosslyn, Va. This material was identified by the late D. N. Shoemaker as *Rheum hybridum* Murr. This is a species of somewhat uncertain botanical status, but there is no reason to doubt that conclusions based on the study of it would apply also to the common cultivated rhubarb (*R. rhabonticum* L.). The growth and development of the planting as a whole were very good for the climatic conditions that prevail in the district.

### METHOD OF SAMPLING

In the first part of the 1932 growing season a series of samples was taken in which every leaf included had been tagged by the time it reached a height of 5 to 7 cm. Leaves were tagged April 11 to 23, and samples were taken 7, 16, 26, 35, 46, and 60 days after the tagging during the period April 18 to June 22. The age of the 70-day-old sample (oldest), however, was estimated; the leaves were selected on July 5 from the healthiest plants and by all signs were the oldest ones in the clump. The blades of practically all these leaves were turning brown and drying at the margin.

The leaves were harvested by pulling them from the crown, as is customary in harvesting rhubarb. The material for analysis was

collected about 10 a. m. on each sampling date, and 30 leaves were harvested for each chemical sample.

The petioles were thinly sliced transversely with a rotary slicer, the mesophyll tissue of the blade was separated from the midrib and most of the large veins, and the material (petiole and leaf-blade tissue separately) was then weighed and immediately put in a fan-driven drier operated at 65° to 70° C.

In 1935 samples were taken from the same plot by the same general procedure, except that tagging dates were scattered throughout the season.

#### GROWTH MEASUREMENTS

The petiole and leaf-blade lengths of 25 leaves of each sample were measured to the nearest 0.5 cm. at the time of sampling.

#### DETERMINATION OF TOTAL DRY MATTER

After the green weight was determined, the samples were immediately put in a fan-driven drier at 65° to 70° C., kept there overnight, then removed, further dried in a vacuum oven for 24 hours at 75° and 27 inches of mercury, and finally weighed from a desiccator to determine the total dry matter. They were then ground in a porcelain ball mill and stored in tightly stoppered brown bottles.

#### BAU'S METHOD FOR OXALIC ACID DETERMINATION

Bau's method of determining oxalic acid (4) involves the precipitation of oxalic acid with a buffer precipitating reagent of calcium acetate, which is called "Kalkessig." It is prepared in the following manner: 330 gm. of crystallized sodium acetate is dissolved in 300 ml. of warm water. After cooling, it is filtered into a 500-ml. volumetric flask. To another 500-ml. flask is added a solution of 25 gm. of crystallized calcium chloride in 50-percent acetic acid; finally enough 50-percent acetic acid is added to make up the volume to the 500-ml. mark. Equal volumes of these two solutions are mixed and allowed to stand at 7° C. or below for 48 hours so that traces of calcium oxalate can deposit. After filtering, this solution is ready for use.

Bau's method for determining oxalic acid in plant tissue is as follows: 15 gm. of fresh plant material is placed in a jar or flask filled with carbonic acid ( $\text{CO}_2 + \text{H}_2\text{O}$ ), and 15 ml. of fuming hydrochloric acid is added to destroy the enzymes and plasmolyze the cells. One hour is allowed for cooling. Then approximately 280 ml. of recently boiled distilled water saturated with  $\text{CO}_2$  is added to the flask; the mixture is agitated frequently for about 14 hours; then the extracting solution is filtered through a hard filter (Schleicher and Schüll No. 602). For precipitation of oxalic acid the clear solution should not contain more than 0.2 percent of it. An aliquot of this solution is then treated in the cold with one-fifth of its volume of the precipitating reagent, set aside for 38 to 40 hours at 7° C., and filtered through a quantitative filter. The filtrate is measured, and the volume of the wash water is also noted. The precipitate is washed with cold water until the chloride test is negative. The paper and precipitate are ashed in a platinum crucible, weighed as the oxide, dissolved in 0.1 N hydrochloric acid, and titrated back with 0.1 N sodium hydroxide. For the calculation of oxalic acid: 1 ml. of 0.1 N acid = 4.40 mg.

## MODIFIED AND IMPROVED METHOD FOR OXALIC ACID DETERMINATION

As stated, the Bau method converts calcium oxalate to the oxide, which is weighed, then dissolved, and titrated. In contrast, the improved method converts calcium oxalate to calcium carbonate, which is the final compound to be weighed. Calcium carbonate is a more satisfactory compound to weigh than calcium oxide, because it is not appreciably hygroscopic.

Bau's method can be simplified, because under certain well-defined conditions the solubility of calcium oxalate in the filtrate and in the wash water can be neglected. Also, as stated on page 40, the recovery of oxalic acid from known solutions is not affected by a fairly wide range in pH; this fact is added assurance that the method applies to moderately great changes in acidity.

All the analyses reported in this study were made on dried rhubarb tissue ground to pass a 30-mesh screen. This was found by experiment to be a prerequisite for complete extraction under the conditions of this investigation. Trial analyses were made by Bau's method (ashing to the oxide), but replicate determinations gave such poor agreement in contrast to those by the improved method, whose precision is shown in table 1, that all the analyses reported in this paper were made by the improved method.

## DESCRIPTION OF IMPROVED METHOD

Dry tissue ground fine enough to pass a 30-mesh screen is weighed out exactly to 1.25, 2.50, or 4.00 gm. (depending on the oxalate content of the tissue) either by the moisture-free method or by some other whereby the moisture is known. The sample is transferred quantitatively into a 250-ml. volumetric flask and made up to about 225 to 230 ml. with approximately 0.25 N hydrochloric acid. This is then heated on a water bath at 70° C. for 45 to 60 minutes, cooled to 25°, and made up to volume with 0.25 N hydrochloric acid. After a thorough shaking, the extract is filtered twice through the same folded filter. If the filtrate is not clear, hydrochloric acid is added to it to precipitate the plant colloids and the mixture is filtered again on a separate filter (usually necessary only in the determination of soluble oxalic acid). Aliquots are immediately taken from this filtrate for determination of total oxalic acid. One or two 100-ml. aliquots are taken from the clear filtrate, put in 400-ml. beakers, and heated just to boiling. Then 20 ml. (or one-fifth the volume of the aliquot) of the calcium acetate reagent is added slowly while stirring to precipitate the oxalate. The mixture is allowed to stand overnight in a refrigerator at 2° to 7°, and upon removal it is filtered immediately through a prepared asbestos mat in a previously ignited and tared Gooch crucible. Any suitable type of filtering crucible will serve provided it will stand a temperature of 550°.

The precipitate is collected in the weighed crucible with the aid of gentle suction and a rubber policeman. It is necessary to wash it 15 to 20 times with small portions of ice-cold distilled water until it gives a negative chloride test. The volume of the total washings should amount to at least 50 to 60 ml.

The precipitate is dried at 100° C. for about an hour and ignited at

500°±25° in a controlled or calibrated electric muffle furnace for about 2½ hours, whereby the calcium oxalate is converted to calcium carbonate. (This conversion is now a preferred procedure for the gravimetric determination of calcium. Willard and Boldyreff (17) have shown this reduction from the oxalate to the carbonate to be quantitative.) The residue, in the form of calcium carbonate,<sup>4</sup> is ready to weigh after it has been cooled in a desiccator. Milligrams of calcium carbonate multiplied by 0.8995 are equivalent to milligrams of anhydrous oxalic acid.

To determine water-soluble oxalic acid in the tissue, distilled water is used instead of 0.25 N hydrochloric acid for the extraction of the oxalates. Because of the presence of pectin and other colloids, it is necessary to make the aliquots 0.25 N with hydrochloric acid, in order to get large and easily filterable calcium oxalate crystals consistently.

#### PREPARATION OF PRECIPITATING REAGENT

By the modified method the oxalic acid is precipitated as the calcium salt by a reagent similar to the one recommended by Bau (4), which he called "Kalkessig." It is prepared as follows:

*Solution A.*—300 gm. of crystalline sodium acetate is dissolved in warm water, cooled, and made to a final volume of 500 ml.

*Solution B.*—25 gm. of anhydrous calcium chloride is dissolved in distilled water, 50 ml.<sup>5</sup> of 50-percent acetic acid is added, and the mixture is made up to a final volume of 500 ml. with distilled water.

Solution A is mixed with solution B, filtered, set in a refrigerator at 2° to 5° C. for 48 hours, and again filtered. The reagent maintains the hydrogen-ion concentration of the precipitating medium at about pH 5.0 to 5.5. Adding 1 part "Kalkessig" to 5 parts of the solution to be tested was found by experiment to be satisfactory and was used throughout the analyses.

#### PREPARATION OF ASBESTOS

The asbestos used in preparing the mats for the Gooch crucibles is thoroughly digested with hydrochloric acid, sodium hydroxide, and nitric acid for several weeks for each treatment. The mats are then washed thoroughly with distilled water to get rid of the fine asbestos that would be washed out and cause a weight loss during subsequent filtration, ignition, and weighing.

#### ASHING TECHNIQUE

A platinum-rhodium thermocouple and potentiometer were used to read the temperature in the electric muffle furnace. It was found that the furnace temperature could be easily held at 500°±25° C. when the

<sup>4</sup> Kolthoff and Sandell (9, pp. 329-331) advocated repeated heating at 500° C. for 30-minute periods to constant weight. In order to test whether there has been decomposition of the calcium carbonate, they moisten the residue, or charge, with 2 or 3 drops of saturated ammonium carbonate solution, allow it to stand at room temperature for 15 minutes, dry it at 110° to 120° for half an hour, and weigh it. There should be no change in weight. If there is an increase in weight, the moistening is repeated with ammonium carbonate and the residue is heated until the weight is constant.

<sup>5</sup> Bau's precipitating reagent used 500 ml. of 50-percent acetic acid. A pH of 3.8 to 4.2, much lower than the pH given by the modified reagent, resulted.

proper rheostat setting was located. Meters that permit watching the temperature at all times are available, and there are still other devices that automatically maintain the furnace temperature at any desired point.

#### EXPERIMENTS TO TEST QUANTITATIVE ACCURACY OF IMPROVED METHOD

Several questions have arisen regarding the strictly quantitative nature of this method when applied to plant tissue under practical conditions of analysis. Is there complete extraction of oxalic acid from the tissue under the conditions chosen for the determination? Are there other relatively insoluble calcium salts present that would cause an error? Is the presence of barium, magnesium, and strontium a source of error? What is the effect of moderate pH changes on precipitation and subsequent recovery of oxalic acid? Does the solubility of the precipitated calcium oxalate seriously affect the accuracy of the final result? Does pectin interfere?

#### COMPLETENESS OF OXALIC ACID EXTRACTION

Dried ground rhubarb tissue was extracted with 1 liter of 0.25 N hydrochloric acid as described on page 37. Completeness of extraction of this material was then tested by adding a second liter of 0.25 N hydrochloric acid to the previously extracted residue and heating for 2 to 3 hours at 70° C. Negative results were obtained from both soluble and total oxalate determinations in a number of test cases with leaf-blade and petiole tissues which were relatively high in oxalic acid. The close agreement of replicate determinations shown in table 1 is further evidence of the uniformly thorough extraction of the material.

#### EFFECT OF OTHER INSOLUBLE CALCIUM SALTS

Table 1 represents a sample record sheet carrying all the details of a typical series of analyses. The volume of the solvent (water or 0.25 N hydrochloric acid) and wash water was so great that other relatively insoluble calcium salts, such as calcium malate and calcium citrate, could be neglected as a contamination of the precipitate. These salts are sufficiently soluble to be removed almost completely by the method used.

#### EFFECT OF INSOLUBLE SALTS OF OXALIC ACID OTHER THAN CALCIUM

Spectrographic examination was made of several calcium carbonate residues set aside from the analysis. Although small traces of magnesium, strontium, and barium were detected in the carbonate residue, the total of these elements would never be above 0.2 percent of the calcium carbonate charge. The residual calcium carbonate compared very favorably in purity with a chemically pure grade of calcium carbonate.

#### EFFECT OF pH OF PRECIPITATION MEDIUM

The effect of the pH of the precipitation medium on the precipitation and recovery of oxalic acid was determined as follows: A solution of oxalic acid the equivalent of 0.200 gm. of calcium carbonate (or

TABLE 1.—*Sample record sheet for total oxalic content of replicate samples of petiole tissue, 1932*

[All analyses reported in this study made by the modified and improved method described on p. 37]

Age of tissue part (days)	Total volume	Weight of sample	Sample in aliquot	Aliquot taken	Wash water	Calcium carbonate recovered	Oxalic acid equivalent	Anhydrous oxalic acid (dry-weight basis)		
								Milliliters	Grams	Percent
7	1,000	2.5	0.5	200	140	31.1	27.97	5.60	5.58	5.70
7	1,000	2.5	.5	200	100	31.0	27.88	5.60	5.58	2.2
7	1,000	2.5	.5	200	55	31.1	27.97	5.60	5.58	
7	1,000	2.5	1.0	400	100	64.5	58.02	5.80	5.90	
7	500	1.25	1.0	400	60	65.5	58.96	5.90		
16	1,000	2.5	1.0	400	95	90.6	81.50	8.15	8.15	.0
16	1,000	2.5	1.0	400	70	90.6	81.50	8.15		
26	1,000	2.5	1.0	400	80	101.2	90.98	9.10	9.05	.27
26	1,000	2.5	1.0	400	80	100.7	90.54			
35	1,000	2.5	1.0	400	65	102.4	92.11	9.21	9.21	.0
35	1,000	2.5	1.0	400	50	102.4	92.11	9.21		
46	1,000	2.5	.5	200	100	61.4	55.23	11.05	11.08	.14
46	1,000	2.5	1.0	400	75	123.2	110.82	11.08		
60	1,000	2.5	1.0	400	75	135.2	121.6	12.16	12.57	.12.37
60	500	1.25	1.0	400	45	139.7	125.7			.12.37
60 <sup>1</sup>	1,000	2.5	.5	200	110	68.6	61.71	12.34	12.08	.12.21
60	1,000	2.5	1.0	400	120	134.3	120.8	12.08		.12.21
70	1,000	2.5	.5	200	100	78.9	70.97	14.19	13.92	13.98
70	1,000	2.5	1.0	400	65	154.8	139.2	13.92		
70	500	1.25	1.0	400	95	153.7	138.3	13.83		

<sup>1</sup> Second lot.

0.1799 gm. of oxalic acid) per liter was prepared in 0.25 N hydrochloric acid and treated with the precipitating reagent at pH 4.40, 4.75, and 5.05. Each hydrogen-ion concentration gave satisfactory precipitation from the standpoint of recovery of from 99.85 to 100.82 percent of the known concentrations, and the replicate determinations agreed reasonably well.

#### EFFECT OF SOLUBILITY OF CALCIUM OXALATE

Bau (4) precipitated calcium oxalate at a temperature not exceeding 7° C. in a cold room and gave the following values for the solubility: 1 liter of filtrate holds in solution the equivalent of 3.42 mg. of anhydrous oxalic acid; 1 liter of wash water holds in solution the equivalent of 4.64 mg. of anhydrous oxalic acid.

In order to determine the solubility of calcium oxalate under the conditions of these analyses, oxalic acid was recovered as before from 2 liters of a pure solution of sodium oxalate of known concentration. When the solubility values of calcium oxalate for the filtrate and the wash water were used in calculating the percentage of recovery, the recovery was found to be from 1.4 to 4.3 percent too high. When the solubility was neglected, however, the percentage of recovery was

found to vary only from 99.85 to 100.82 percent of the known value.

The technique followed in the use of the precipitant affords a possible reason why the solubility values need not be considered in this work. Bau's reagent always was allowed to stand several days in loosely stoppered bottles and then was filtered through a quantitative filter just before use. Bau (3) found that ordinary acetic acid may contain glyoxylic acid, which is oxidized by air to oxalic acid. Another source of oxalic acid as an impurity is the sodium acetate. Therefore, these two chemicals may contain enough oxalic acid to keep the reagent saturated with respect to calcium oxalate before it is used and this saturation tends to make the results too high. Furthermore, if the precipitate is washed rather rapidly with ice-cold water with the aid of suction and if the volume of washings and filtrate are kept to a minimum, at a temperature of about 5°C., the small amount of calcium oxalate dissolved is negligible. Calculations made of the ultimate solubility of  $\text{CaC}_2\text{O}_4 \cdot \text{H}_2\text{O}$  in 100 ml. of water at 5° for three equivalent calcium carbonate recoveries, 25 mg., 50 mg., and 100 mg., amount to 1.64, 0.82, and 0.41 percent, respectively. Wash-water losses of calcium oxalate actually would be much lower than this because of the rapid washing with the aid of suction.

#### EFFECT OF PRESENCE OF PECTIN

It was difficult to get good calcium oxalate crystals from 0.5-percent pectin solutions, but concentrations of pectin in solution up to 0.4 percent did not influence the accuracy of the method under the conditions specified. The plant material under consideration contained no troublesome amounts of pectin when handled as described.

#### RESULTS OF ANALYSES

The 1932 samples (fig. 1 and table 2) show a pronounced increase

TABLE 2.—Variation in oxalic acid content of rhubarb tissue with age, 1932

Tissue and its age (days)	Total height of leaf	Total dry matter	Anhydrous oxalic acid			
			Soluble (in water)		Total (soluble in hydrochloric acid)	
			Fresh-weight basis	Dry-weight basis	Fresh-weight basis	Dry-weight basis
Leaf blades:						
7	13.9	16.87	0.14	0.83	0.24	1.42
16	32.7	12.84	.226	1.76	.299	2.33
26	49.8	11.50	.454	3.95	.542	4.72
35	63.0	10.95	.529	4.82	.637	5.82
46	83.5	11.73	.890	7.59	1.22	10.40
60	85.6	10.41	.960	9.23	1.40	13.47
70		11.04	.947	8.57	1.63	14.74
Petioles:						
7		6.0	6.00	.237	3.95	.342
16		17.7	4.95	.322	6.5	.403
26		27.8	5.74	.386	6.73	.521
35		36.6	5.53	.375	6.78	.509
46		46.6	6.64	.422	6.36	.734
60		44.6	6.71	.426	6.35	.830
70			6.75	.482	7.15	.944

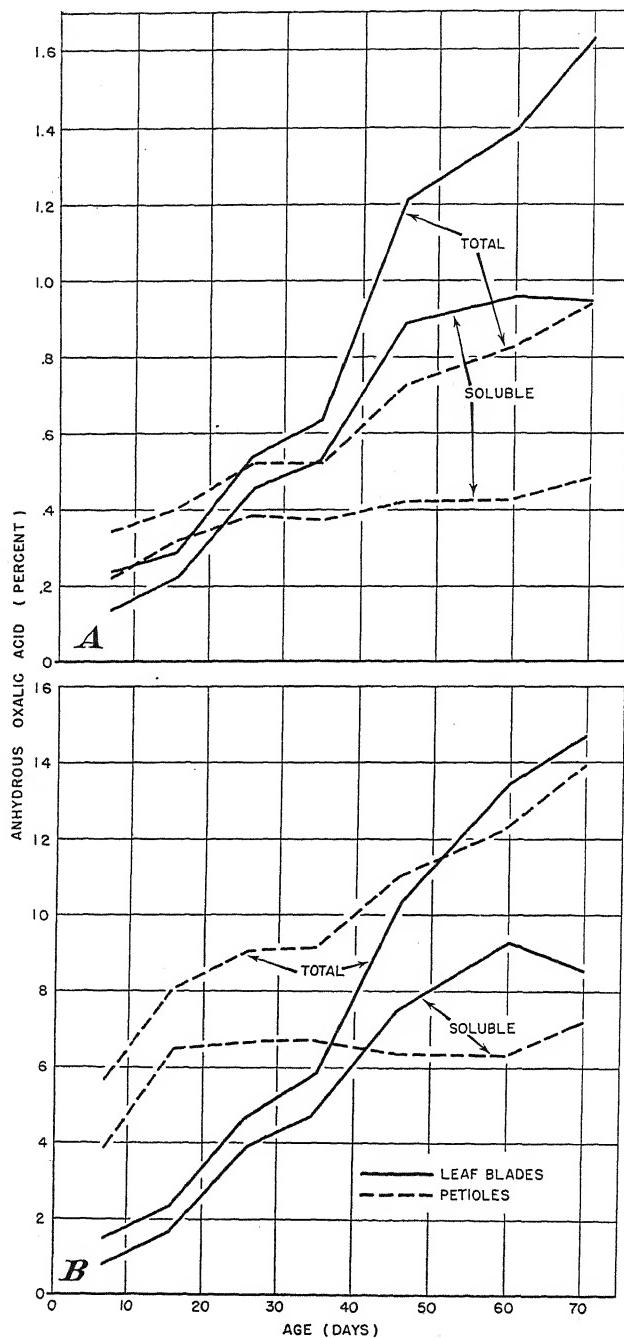


FIGURE 1.—Oxalic acid content of rhubarb leaf blades and petioles of different ages, 1932: A, Fresh-weight basis; B, dry-weight basis.

in concentration of total and soluble oxalates with age of the tissues. This situation is also shown to some extent for the soluble oxalate content in the 1935 series (table 3).

TABLE 3.—*Variation in soluble oxalic acid content of rhubarb tissue with age, 1935*

Age of tissue (days)	Total height of leaf	Total dry matter	Anhydrous oxalic acid in —			
			Leaf blades		Petioles	
			Fresh-weight basis	Dry-weight basis	Fresh-weight basis	Dry-weight basis
			<i>Centimeters</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>
1.....	20.1	17.57	0.302	1.72	0.426	2.42
7.....	43.8	14.38	.311	2.16	.419	2.91
10.....	56.1	16.28	.496	3.05	.503	3.09
29.....	47.9	16.94	1.15	6.79	.523	3.09
35.....	47.5	14.76	1.22	8.26	.494	3.34
54.....	60.8	16.17	1.25	7.73	.361	3.47
65.....	62.3	15.46	.945	6.11	.572	3.70
70.....	72.0	15.52	1.02	6.57	.532	3.43

The petiole tissue up to 16 days of age showed a consistently higher concentration of both total and soluble oxalic acid than leaf-blade tissue (fresh-weight basis) in the 1932 series of samples and of the soluble acid in the young samples of the 1935 series (tables 2 and 3). When the stalks had reached full maturity (leaves 46 to 70 days old), the oxalic acid content of the petioles was about one-half that of the leaf blades for both total and soluble acid in all the determinations made. A narrower range in concentration of soluble and total oxalates is indicated for the petioles than for the leaf blades in all the material investigated. In the 1932 series, the mature leaf blades contained six or seven times as much oxalic acid as the young ones, whereas the petioles contained only two or three times as much.

The 1935 series (table 3) had roughly a content of 1.2 gm. of soluble oxalate per 100 gm. of fresh leaf-blade tissue for the 29- and 35-day samples. According to this analysis, one-half pound of leaf blades of either of these two samples would contain 2.7 gm. of soluble oxalic acid. It would take roughly a pound of petioles of somewhat older tissue (54 days or over) to contain an equivalent amount of soluble oxalate. Samples of rhubarb taken in other seasons immediately after a period of high photosynthetic activity might, of course, run higher in soluble oxalates than those shown in table 3. Van Itallie and Lemkes (7) found that the literature shows the fatal dose of oxalic acid to be 2 to 5 gm., but these writers thought that it was much lower. Kohman (8), by summing up much of the literature on this subject, found that the fatal dose ranges from 2 to 30 gm. He gave 10 gm. as the fatal dose for adults and 3 to 4 gm. for children.

#### DISCUSSION

Culpepper and Caldwell (6) made titratable-acidity determinations on rhubarb with as many as five series of samples taken as described herein. They found that the shape of the titratable-acidity curve

was in general the same throughout the season, differing only slightly in intensity in midseason or during the period of most active photosynthesis.

Previously no one had determined oxalates on such a series of samples having known ages and showing increasing stages in maturation. Other workers have analyzed rhubarb tissue for oxalic acid content, but they had not systematically investigated content in relation to age.

In this work Bau's procedure (4) was modified by ashing the calcium oxalate to the carbonate rather than to the oxide and certain improvements of technique which have given more reliable results than the older method were introduced. Although comparatively large volumes of extracting solution were used, experience has shown that smaller volumes can be used successfully. This means that it is possible to take, say, 2.0 to 2.5 gm. or any other suitable dry weight of sample, make it up to volume in a 250-ml. flask, and use two 100-ml. aliquots for the analysis if duplicate determinations are desired. Even the use of smaller volumetric flasks would serve for many problems when great sensitivity is not needed.

Aside from the error introduced by ashing the oxalate to the oxide, Bau's method of analysis has fallen into some disrepute because of certain ascribed sources of error supposedly inherent in the direct application to plant tissue. On the surface these supposed errors seem very important, yet when optimum conditions were found for carrying out the analysis there was no interference of any serious magnitude with the direct quantitative determination of oxalic acid in rhubarb tissue.

The most serious error which the procedure might possibly have would be due to the loss of oxalates upon improper drying of the fresh tissue that would favor enzymic changes in succulent material such as rhubarb petiole. In this work that possibility was minimized by slicing the material into thin sections and by the use of a fan-driven drier. Some analyses made of fresh tissue would suggest that a very slight loss had occurred in one or two cases with petioles, but other replicates were in good agreement.

Nearly all of the modern methods of analysis of plant tissue for organic acids employ ether extraction of the tissue as the first step; subsequently the separate organic acids, such as malic, citric, tartaric, and oxalic, are determined on the aliquots from the water extract of the ether residue. These methods are now highly refined and capable of yielding valuable data on acid metabolism in growing plants. Pucher, Wakeman, and Vickery (13) have perhaps done more for the development of sound analytical methods than any other group of workers in the organic acid field. Kohman (8, p. 102) stated: "There is great need for a reliable method for the accurate determination of oxalic acid in foods." It appears that when several organic acids must be determined the methods of Pucher et al. are the most reliable now available and should, therefore, be employed for detailed precision work. However, there are certain problems involving plants used as food, where there is a need for a less time-consuming method, specifically for oxalic acid, that is both reliable and reasonably accurate.

## SUMMARY AND CONCLUSIONS

A direct and reliable modification of Bau's method (4) for the determination of oxalic acid in plant material by aqueous extraction of the ground dried tissue is described. In the use of this method it is important that succulent material be carefully prepared and dried under the proper conditions in order to avoid the enzymic loss of oxalates.

Certain sources of error believed by many workers to invalidate the usefulness of Bau's method have been investigated by the writer and found to be without appreciable influence on the quantitative recovery of oxalates from rhubarb (Ruby variety) tissue when suitable precautions in technique were adopted. Completeness of extraction of the tissue was assured by negative tests for oxalates when the residue was repeatedly extracted with hot water and dilute hydrochloric acid and by agreement of replicate determinations. Modifications of technique that make for reliability of the method were (1) ashing the calcium oxalate to calcium carbonate at  $500^{\circ} \pm 25^{\circ}$  C. and weighing and (2) washing rapidly with ice-cold distilled water, using suction and a minimum volume of wash water and filtrate, which eliminates the need for taking solubility of calcium oxalate into account.

Pectin from rhubarb tissue and a purified commercial pectin in solution did not interfere with recovery; a relatively wide variation in pH (4.40 to 5.05) had no effect on the accuracy of the analysis.

The improved method was used to study the concentration of total and soluble oxalic acid in rhubarb leaf samples. These samples consisted of leaf blades and petioles of known ages; each sample of a particular age was composed of a minimum of 30 tagged individual leaves. Data were obtained representing equivalent anhydrous oxalic acid in water-soluble and hydrochloric-acid-soluble forms in average leaf blades and petioles of different known ages.

In the 1932 series the concentration of water-soluble oxalates of 26- to 46-day-old fresh rhubarb of market size was approximately 0.39 to 0.42 percent in the petioles and 0.45 to 0.89 percent, respectively, in the leaf blades. The 1935 series suggests what appears to be a seasonal march or increase in oxalates in the plant as the season advances. More analyses should be made on samples like the 1932 series taken three or four times during the growing season.

This study of the oxalate content of rhubarb leaves of different ages serves as an aid in determining the best time to harvest rhubarb for market. From these data it appears that rhubarb with leaves from 10 to 35 days old is best from the standpoint of eating quality and soluble oxalate content. However, there was not much increase in soluble oxalate in the edible stalk (petiole) of leaves 46 to 60 days old over that of those 35 days old.

The oxalate content of the leaf blade exhibited a more pronounced increase with age than that of the petiole. This was true of both soluble and total oxalates. The soluble oxalic acid in the petiole was initially higher in the young samples and went through only a very gradual increase as the petiole attained maturity. In the leaf blade the concentration of the water-soluble acid was lower initially, but at

full maturity (leaves 46 to 70 days old) its content was roughly double that found in the petiole on a fresh-weight basis.

In the 1932 series the concentration of both soluble and total oxalic acid was greater in the petiole tissue than in the corresponding blades for samples up to and including 16-day-old leaf blades.

The data obtained in this investigation support the idea expressed by Allsopp (1) and others that the continuous increase in acids in the leaf blades during the season of most active photosynthesis suggests that they arise either as a direct result of photosynthesis or indirectly from carbohydrates. The magnitude of the concentration changes alone in the leaf blades certainly makes it clear that the acid arises in a synthetic process involving photosynthesis.

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# STAMEN MORPHOLOGY IN FLOWERS OF THE MUSKMELON<sup>1</sup>

By D. M. MCLEAN

Research assistant in plant pathology, Michigan Agricultural Experimental Station

## PREVIOUS OBSERVATIONS

The morphological interpretation of the dissimilar stamens which constitute the androecium of certain members of the Cucurbitaceae has been a controversial matter practically since the taxonomic description of the family was published. Recently, Heimlich (*4*)<sup>2</sup> described the androecium of cucumber (*Cucumis sativus*) as consisting of two complete stamens and one "half stamen" in which one theca (pollen sac) fails to develop, reaffirming the original interpretation of Naudin (*7, 8*) that the tetrasporangiate stamens are normal, while the smaller bisporangiate stamens (half stamens) had resulted either from the abortion of the missing half or else from the splitting of a tetrasporangiate stamen in two. Judson (*5*) refers to only three staminodia in the carpellate flower of cucumber. Miller (*6*), comparing *Echinocystis lobata* with other genera, concluded that the bisporangiate stamen in Cucurbitaceae is a complete one and not a half organ in the developmental sense. He (*6, p. 277*) regards *Cucumis* as having "five stamens, four of which are united in two masses," and agrees with Payer (*9, pp. 440-446*), Eichler (*3*), and Baillon (*1, 2*), who consider the tetrasporangiate stamen as a double organ. Miller (*6*) based his interpretation upon the observation that the tetrasporangiate stamen in *Echinocystis* has its origin in two separate primordia which form a single structure owing to the lateral coalescence and the growth of intervening tissue between the original primordia, and on the further fact that staminodia in the carpellate flower, although disposed like stamen primordia, commonly develop without combining in pairs. Thus, the members of the genus *Cucumis* are generally considered to be trimerous, at least in respect to the androecium, and according to Rosa (*10*), this condition is usually accompanied by a tricarpellate ovulary surmounted by a three-lobed stigma. Rosa (*10*) found two varieties of melons, the Golden Beauty casaba (*Cucumis melo* var. *inodorus* Naud.) and Pomegranate (*C. melo* var. *odoratissimus* Naud.) to be pentamerous both as to androecium and gynoecium. There are five single stamens (like the half stamen of other Cucumi) in both the stamineate and perfect flowers, and there are five separate locules in the ovularian cavity.

<sup>1</sup> Received for publication April 13, 1945. Journal article No. 784 from the Michigan Agricultural Experiment Station.

<sup>2</sup> Italic numbers in parentheses refer to Literature Cited, p. 54.

Most of these investigations have been made on plants of a monoecious character. In monoecious species, the carpellate flowers frequently bear staminodia but only rarely do these structures become functional stamens. Some genera have staminodia in the carpellate flower equal in number to the stamens in the staminate flower; other genera have staminodia in the carpellate flower unequal to the stamens in the staminate flower.

Almost all varieties of commercially cultivated muskmelons (*Cucumis melo*) show a modification of the monoecious characteristic which is termed andromonoecious. Andromonoecious plants bear perfect flowers in addition to carpellate ones and staminate ones. Rosa (10) found several exotic varieties of European and Asiatic origin which produced carpellate and staminate flowers but no perfect flowers. On the andromonecious plants of some varieties of *C. melo*, occasional carpellate flowers have been observed. The occurrence of these aberrant sex forms seems to be more common on plants cultured in the greenhouse. Rosa (10) observed 15 instances of carpellate flowers in 2,802 apparently perfect flowers, or 1 in 187, indicating that about 1 plant in 3 may produce a single flower of aberrant sex form. He could not correlate the occurrence of aberrant forms with any particular environmental condition, or with any certain stage in plant development.

In muskmelon breeding it is frequently necessary to emasculate the stamens from the perfect flowers. This procedure necessitates the examination of many flowers. In several commercial varieties and certain selections from inbred lines, variation from the usual "two complete and one half stamen" situation has been frequently noted in perfect flowers. Occasionally five bisporangiate individual stamens are observed. More frequently three bisporangiate and one tetrasporangiate stamen comprise the androecium. Such variation may likewise be observed in staminate flowers, though not so commonly. According to Rosa (10) most present-day varieties of melons present a trimerous gynoecium; however, both canteloups and watermelons occasionally produce fruits with four carpels. These observations suggested that the trimerous androecium of muskmelon flowers, which is now the prevailing type in most varieties, probably arose from the pentamerous by two bisporangiate stamens having united to form the tetrasporangiate stamen which seems to be a double organ. A study of the vascular anatomy of staminate and carpellate flowers was made to aid in the proper interpretation of the stamen arrangement.

#### STAMEN ARRANGEMENT

The androecium of the staminate flower of muskmelon usually consists of two groups of paired stamens and a single stamen. The paired stamens have, in each case, two vermiform or S-shaped thecae or "pollen sacs," but the single stamen has only one. Each theca results from the dehiscence of two microsporangia. The paired stamens adhere to each other by their connectives which prolong above the stamens. These prolongations are forked, indicating the double characteristic of the structure. The single stamen has only a single prolongation of the connective. Where this arrangement is observed

usually only three short filaments are apparent. The filaments diverge from the receptacle at the base of the inner surface of the perianth tube.

The stamen arrangement and development in the perfect flower may be the same as in the staminate flower, but frequently differs from the usual situation in the staminate flower and constitutes a clue to the homology of the individual stamens. Perfect flowers have been observed which contained five bisporangiate stamens. It seems obvious that the tetrasporangiate and bisporangiate organs cannot be homologous, for while one is a simple stamen, the other two, being made up of two single stamens, are compound.

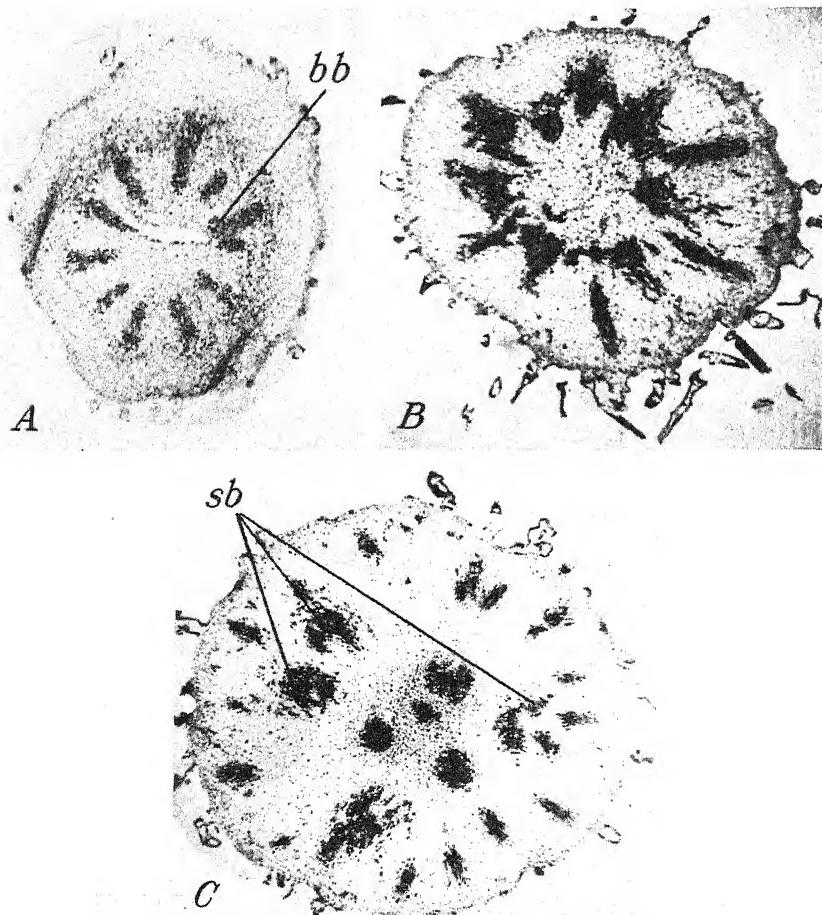


FIGURE 1.—*A*, Transverse section through a pedicel showing 10 main vascular bundles; 1 bundle has branched (*bb*).  $\times 50$ . *B*, Transverse section through the receptacle of a staminate flower just above the vascular plate, showing 2 cycles of vascular bundles that traverse the perianth tube.  $\times 36$ . *C*, Transverse section taken  $240 \mu$  nearer the perianth tube than *B*. Centripetal branches (*sb*) from bundles which supply the perianth extend to the stamens.  $\times 41$ .

The vascular structure of the staminate and perfect flowers was investigated with particular reference to the vascular supply of the stamens.

#### VASCULAR CONTINUITY

The flower pedicel is traversed by 10 main vascular bundles (fig. 1, A). Before the pedicel joins the receptacle several bundles may branch, which usually results in 12 bundles with anastomosing branches which form a vascular plate at the base of the receptacle cup. From this plate usually 10 main bundles ascend obliquely through the receptacle and supply the perianth (fig. 1, C). These bundles, retaining their identity, extend outward and upward toward the sepal and petal lobes (fig. 1, B, C.). Between adjacent bundles in the perianth tube usually smaller bundles arise which likewise extend toward the petals and sepals from the vascular plate. In the perianth

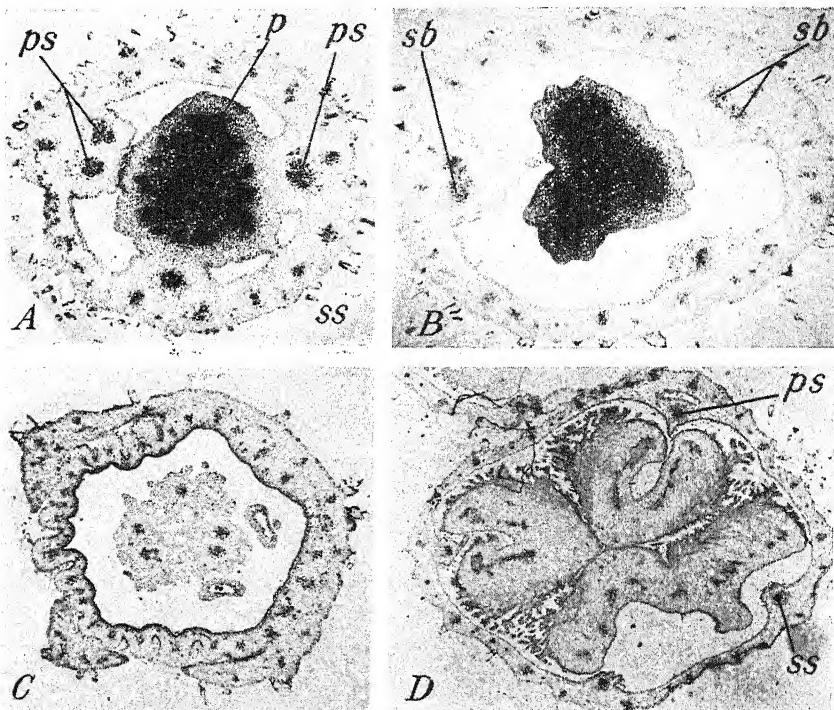


FIGURE 2.—*A*, Transverse section taken about 250  $\mu$  from figure 1, *C*, showing vascular bundles (*ps*) which supply paired stamens and a single bundle (*ss*) which supplies the unpaired stamen; one double bundle appears as a single bundle; (*p*) pistillodium.  $\times 34$ . *B*, Transverse section through another staminate flower at about the same level, showing the true identity of the paired double vascular bundles (*sb*).  $\times 36$ . *C*, Transverse section through a staminate flower just above the stamens, showing the vascular bundles that extend through the prolongations of the connectives.  $\times 26$ . *D*, Transverse section through a carpellate flower which contained three bisporangiate stamens containing a single bundle each and one tetrasporangiate stamen. The vascular bundles (*ps*) supplying the paired stamens appear like a single bundle; *ss*, a single bundle.  $\times 14$ .

tube the main bundles form two cycles (fig. 1, *B*, *C*). The bundles of the outer cycle terminate in the sepal lobes and alternate with the bundles of the inner cycle. Centripetal branches from the bundles of the inner cycle supply the stamens, while the main bundles lead to the petal lobes (fig. 1, *C*).

As shown by Heimlich (4) and Judson (5) in cucumber, and by Miller (6) in *Echinocystis lobata*, the sepal primordia and petal primordia arise in different cycles. Where double stamens occur in the androecium of *Cucumis melo*, each of the individual stamens arises in separate primordia, and the vascular bundles traversing these stamens may be traced to adjacent bundles within the cycle (fig. 1, *C*, and fig. 2, *A*, *B*). In transverse sections, the bundles supplying paired stamens sometimes appear as a single bundle (fig. 2, *A*, *D*). Where the vascular elements are so closely united, it appears that they may branch from adjacent bundles of the different cycles in the perianth tube (fig. 1, *C*, and fig. 2, *A*, *B*) ; i. e., from adjacent bundles supplying members of the calyx and corolla. When the stamens are not paired and each stamen retains its individuality in the androecium they are homologous and their vascular continuity may be traced to vascular bundles of the inner cycle in the perianth tube (fig. 2, *D*).

Each stamen is supplied by a single bundle (fig. 1, *C* and fig. 2, *A*, - *D*). The bundles are unbranched except near the distal end of the stamen where the branches extend to the microsporangia. Each bundle continues throughout the prolongation of the connectives. (fig. 2, *C*).

#### DISCUSSION

Miller (6) has shown that the tetrasporangiate stamen in *Echinocystis lobata* has its origin in two primordia that are distinctly separate and he concludes that this structure is double. Baillon (1) and Payer (quoted by Baillon) describe the same condition in *Bryonia*, indicating that the larger stamens are double.

The common occurrence of three and sometimes five individual bisporangiate stamens in *Cucumis melo* strongly suggests the double nature of tetrasporangiate stamens. The carpellate flower of *Fervillea*, according to Baillon, has five staminodia which correspond to the five bisporangiate stamens in the staminate flower. *Bryonia* also has five staminodia in the carpellate flower, but only three staminate masses in the staminate flower. As shown by Miller (6), *Echinocystis* reveals a similar situation—two staminate masses (three stamens) in the staminate flower and three staminodia of which two are paired, in the carpellate flower. *Thladiantha*, as described by Tison (quoted by Baillon) clearly shows the homology between the five separate bisporangiate stamens, but four of them are arranged in two pairs and although not united, stand close together. The fifth is isolated without a mate. This condition is not very different from that in *C. melo*.

While one may agree with Heimlich (4) that the number of vascular bundles in an organ cannot be regarded as proof of its homology, the presence of two bundles in each tetrasporangiate of *Cucumis melo* can hardly be interpreted in any other manner.

The bisporangiate condition of individual stamens in *Cucumis* as contrasted with the usual tetrasporangiate stamens in most angiosperms cannot be given great consideration in settling homologies, for variation exists in different families. Miller (6) has pointed out that from what may be regarded as a single microsporangium in the stamen of *Lemna* there is a progression up to large numbers of microsporangia in the branched stamens of such forms as *Ricinus* and *Calothamnus*, and bisporangiate stamens is the common situation in the *Asclepiadaceae*.

On the basis of the evidence presented, it seems probable that the flowers of muskmelon (*Cucumis melo*) and perhaps all of the Cucurbitaceae were originally pentamerous throughout. However, most present-day varieties of melons present a trimerous androecium and, according to Rosa (10), a trimerous gynoecium.

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## VARIATIONS IN THE CHEMICAL COMPOSITION AND INSECTICIDAL PROPERTIES OF THE YAM BEAN (*PACHYRRHIZUS*)<sup>1</sup>

By ROY HANSBERRY, formerly *associate professor of insect toxicology*, ROBERT T. CLAUSEN, *associate professor of botany*, and L. B. NORTON, *assistant professor of insecticidal chemistry*, New York (Cornell) Agricultural Experiment Station<sup>2</sup>

### INTRODUCTION

After the toxicity of the seeds of the yam bean to several species of insects had been established (3)<sup>3</sup>, studies were made on the taxonomy of the genus (1) and on the chemical components of the typical species (*Pachyrrhizus erosus* (L.) Urban) (6, 7). In the present paper some of the relationships between these various phases of the problem are reported.

### MATERIALS

The samples upon which this study is based were collected in Mexico, Guatemala, and El Salvador during the late summer of 1943, or were sent to the laboratory for insecticidal and chemical evaluation by various individuals or agencies interested in the potential insecticidal value of the yam bean.

A detailed description of the various species is found in the taxonomic report of Clausen (1). *Pachyrrhizus erosus* is the common yam bean cultivated widely in Mexico for its edible watery sweet tubers. The samples of this species represent different varieties from various soil types and elevations. *P. strigosus* Clausen is a small-seeded species, which fact together with its limited distribution, would probably restrict its economic value as a source of insecticidal material. *P. tuberosus* (Lam.) Spreng. is a South American species with large seeds and large leaves, tubers, and vines. *P. ahipa* (Wedd.) Parodi is also a South American species, cultivated in Bolivia and in northern Argentina. It has plump seeds similar in shape to those of *P. tuberosus* but smaller. Material of the other two known species, *P. panamensis* Clausen and *P. vernalis* Clausen, has not been available in sufficient quantity for insecticidal and chemical analyses.

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<sup>3</sup> Italic numbers in parentheses refer to Literature Cited, p. 64.

## CHEMICAL METHODS

The samples were ground in a laboratory Raymond mill, extracted with chloroform, and the amount of oil and resin determined in the extract. Fluorescence measurements and several quantitative colorimetric tests proposed for rotenone determination were made on the resin fraction. Similar tests on the seven pure compounds previously isolated (7) showed sufficiently different responses (table 1) to permit estimation of the approximate amounts of these compounds in each sample.

The extractions and separations of oil and resin fractions were made on single samples. All color tests were made in duplicate on aliquots from the resin fractions. If duplicates failed to check within about 5 percent, further pairs of aliquots were run. If results were still inconsistent, or were unusually high or low for one or more of the constituents, a fresh sample was extracted and the complete analysis repeated. Where a color test showed an unusual intensity, it was repeated at a different dilution to bring the light transmission into a more reliable range (30-80 percent).

TABLE 1.—*Results of colorimetric, fluorometric, and insecticidal tests of pure compounds isolated from the yam bean (7)*

Test	Compound No.						
	I	II	III	IV (rotenone)	V (erose- none)	VI	VII (pachyrrhizid)
Goodhue test (520 m $\mu$ ) <sup>1</sup> .....	0	20	0	100	20	0	0
Meijer test (520 m $\mu$ ) <sup>1</sup> .....	0	100	37	100	100	18	83
Meijer test (430 m $\mu$ ) <sup>1</sup> .....	0	136	133	100	126	86	122
Rogers-Calamarie test (590 m $\mu$ ) <sup>1</sup> .....	0	107	0	100	136	0	24
Fluorescence (365-m $\mu$ filter for exciting light).....	{ Intense; maximum at 410 m $\mu$ .	Slight	{ Intense; maximum at 480 m $\mu$ .	Slight	Slight	Slight	Slight
Toxicity to Mexican bean beetle.....	None	None	None	Toxic	Slight	None	Slight
Toxicity to silkworm.....	Slight?	Slight?	Slight?	Toxic	Toxic	None	Toxic

<sup>1</sup> As percent rotenone giving equivalent color.

## EXTRACTION AND SEPARATION OF OIL AND RESIN

A 5-gm. sample of coarsely ground yam beans was extracted in a Soxhlet extractor with chloroform for 24 hours, the defatted marc dried and reground in a glass mortar, and the extraction continued for an additional 24 hours. Most of the solvent was distilled from the extract, and the remainder was removed by warming under reduced pressure until all foaming ceased.

The residue was taken up in 25 ml. of petroleum ether and 10 ml. of nitromethane, shaken in a separatory funnel, and the lower nitromethane layer drawn off. The petroleum ether layer was extracted with two more 10-ml. portions of nitromethane. The combined nitromethane extract was washed with one portion of fresh petroleum ether, which was added to the original petroleum ether solution.

The nitromethane solution was filtered, concentrated to a small volume under reduced pressure, and the last of the solvent removed by warming under a higher vacuum. The residue was weighed as the

resin fraction. The petroleum ether solution was treated similarly, and the residue weighed as the oil fraction. Heating was kept at a minimum during the removal of the solvent to avoid decomposition.

A light-brown insoluble powder appeared during the separation, and was removed by filtration of the fractions. It was discarded, since it showed no toxicity. The amounts of oil and resin extracted by several different solvents were substantially constant, whereas the amounts of this inert powder varied widely with the solvent. For this reason a direct weighing of the original total extract was not considered significant, and was omitted.

No further tests were made on the oil fraction, which showed no notable toxicity or response to color tests. The resin fraction, which contained practically all of the toxic material, was dissolved in acetone and diluted with acetone to 50 ml. Ten ml. of this solution was further diluted to 100 ml. with acetone to give the "stock solution" used for all of the subsequent tests.

#### FLUORESCENCE MEASUREMENTS

The acetone was removed from 0.2 ml. of the stock solution by warming under reduced pressure. The residue was dissolved in 10 ml. of redistilled benzene. Standards were prepared containing 0.1 microgram of compound I per milliliter of benzene and 1.0 microgram of compound III per milliliter of benzene respectively. A Coleman Photofluorometer with a 365-m $\mu$  filter for the exciting light and a 410-m $\mu$  filter for the fluorescent light was adjusted to read 0 for a benzene blank and 100 for the compound I standard. The compound III standard and the unknowns were then read. With the sensitivity setting left at the same point, the 410-m $\mu$  filter was replaced by a 480-m $\mu$  filter, the benzene blank again set to 0, and both standards and the unknowns read again. Any unknown showing a fluorescence stronger than the standards was diluted with a known volume of benzene, because the direct proportionality between fluorescence and concentration no longer held at higher concentrations. Under the specified conditions, the fluorescence given by each of the two compounds was directly proportional to its concentration, and the relative intensity at the two different wave lengths varied sufficiently to permit separate calculations of the concentrations of compounds I and III.

The equations for these calculations were derived from the standardization as follows: where  $F_{410}$  and  $F_{480}$  represent the numerical readings on the fluorometer scale with the two filters designated, and the symbols (I) and (III) represent the percentages of compounds I and III respectively in the original sample:

Standardization:	$\gamma/\text{cc.}$	$F_{410}$	$F_{480}$
Compound I-----	0.1	100	1.5
Compound III-----	1.0	45	30

With the sample and aliquot used, the percentage of each constituent in the original material is numerically equal to one-half the number of micrograms per cubic centimeter found in the aliquot, so that

$$F_{410} = 2,000 (\text{I}) + 90 (\text{III}) \quad (\text{a})$$

$$F_{480} = 30 (\text{I}) + 60 (\text{III}) \quad (\text{b})$$

Multiplying (b) by 1.5 and subtracting,

$$F_{410} - 1.5 F_{480} = 1,955 \quad (I)$$

$$(I) = .00051 (F_{410} - 1.5 F_{480}) \quad (c)$$

and from (b)

$$(III) = .017 (F_{480} - 30) \quad (I) \quad (d)$$

Equations (c) and (d) were used for the final calculations, with the constants adjusted where necessary for variations in the standardization data.

#### MEIJER TEST<sup>4</sup>

The original Meijer test (5) was modified by the use of an acetone solution directly without dilution with water. A 0.5-ml. portion of the stock solution was pipetted into a 22-mm. matched test tube and 15.5 ml. of a solution of 100 mg. of sodium nitrite in 1 liter of concentrated sulfuric acid was added from a special pipette, with constant shaking. The tube was immediately placed in a bath at 20° C. After 1 hour the colors were read against a blank prepared at the same time in a Lumetron model 402E photoelectric colorimeter with filters of both 520 m $\mu$  and 430 m $\mu$ . The "rotenone equivalent" for each wave length was determined from calibration curves prepared with known amounts of pure rotenone.

#### GOODHUE (2) TEST

A 3-ml. portion of the stock solution was pipetted into a colorimeter test tube. Three milliliters of a freshly prepared mixture of 1 volume of potassium hydroxide (40 gm. per 100 ml. of water) and 7 volumes of sodium nitrite (1 gm. per liter of 95 percent alcohol) were added, the tube shaken, and placed in the 20° bath. After exactly 5 minutes, 7.5 ml. of dilute sulfuric acid (1: 3) were added, and the tube was shaken well and replaced in the bath. After 45 minutes, 15 ml. of ether were added, and the tube was shaken vigorously and replaced in the bath. Fifteen minutes later, the color in the lower layer was read against a blank developed for the same length of time, in the Lumetron colorimeter with a 520-m $\mu$  filter. The "rotenone equivalent" was determined from a calibration curve prepared with pure rotenone.

#### ROGERS-CALAMARI (8) TEST

Although Jones (4) reported that the form of the Rogers-Calamari test carried out in acetone solution did not appear to give good quantitative results, preliminary tests showed fully as good results as the other forms if special precautions were taken to keep times and temperatures strictly constant, to keep the solutions from strong light, and to compare the colors with blanks developed for exactly the same time. The latter was especially important, as the blanks change rapidly with time.

A portion of the stock solution was diluted with an equal volume of acetone, and a 5-ml. portion of the diluted sample pipetted into a colorimeter test tube. Five ml. of a 10-percent solution of thymol in acetone was added, and the tube left for 15 minutes in the 20° bath.

<sup>4</sup> Sometimes referred to as the Meyer test.

Then 0.1 ml. of 3-percent hydrogen peroxide and 5 ml. of concentrated hydrochloric acid were added as rapidly as possible, and the tube shaken for exactly 1 minute and replaced in the bath. Exactly 45 minutes after the addition of the acid, the color was read against a blank developed for the same time, in the Lumetron colorimeter with a 590-m $\mu$  filter. The "rotenone equivalent" was determined from a calibration curve prepared with pure rotenone.

#### CALCULATION OF INDIVIDUAL COMPOUNDS

The calculation of the quantities of compounds I and III from the fluorescence data has already been described. Calculation of the other compounds was made from the colorimetric data, based on their different responses to the various color tests as shown in table 1. The simplifying assumption was made that the contribution of compound VII to the Rogers-Calamari test was negligibly small. The colorimetric response of compounds II and V was too similar to permit separate determination, and average values were assumed for their colors and only their sum calculated. The following equations were derived for the calculations, where  $M_{520}$  and  $M_{430}$  represent the rotenone equivalents of the unknowns in the Meijer test at the designated wave lengths,  $G$  that in the Goodhue test, and  $R$  that in the Rogers-Calamari test, all in percentage of the original beans. The Roman numerals represent the percentage of the designated compound in the original beans.

I Determined by fluorescence

III Determined by fluorescence

Then, from table 1,

$$R = 1.2 (II + V) \text{ (avg.)} + (IV) + 0.24 \text{ (VII)} \quad (\text{a})$$

$$G = 0.2 (II + V) + (IV) \quad (\text{b})$$

Subtracting (b) from (a), and neglecting the contribution of VII,

$$(II + V) = R - G \quad \text{Equation 1}$$

Rearranging (b),

$$(IV) = G - 0.2 (II + V) \quad \text{Equation 2}$$

From table 1,

$$M_{430} = 1.31 (II + V) \text{ (avg.)} + 1.33 (III) + (IV) + 0.86 (VI) + 1.22 \text{ (VII)} \quad (\text{c})$$

$$M_{520} = (II + V) + 0.37 (III) + (IV) + 0.18 (VI) + 0.83 \text{ (VII)} \quad (\text{d})$$

Eliminating (VII) from (c) and (d) and solving for (VI),

$$(VI) = 1.67 M_{430} - 2.45 M_{520} + 0.27 (II + V) - 1.32 (III) + 0.78 (IV) \quad \text{Equation 3}$$

Solving (d) for VII,

$$(VII) = 1.20 [M_{520} - (II + V) - 0.37 (III) - (IV) - 0.18 (VI)] \quad \text{Equation 4}$$

#### SOURCES OF ERROR

The most serious source of error was the unavoidable omission of the unknown constituent probably present in the resin (?) which may account for a substantial part of the toxicity. Since this substance was not isolated in a pure state, its response to the color tests could not be determined.

Neglect of the Rogers-Calamari color given by compound VII would lead to high values for II+V and slightly low values for IV.

The values for VI and VII are considerably less certain than the others. Their calculation involves the results of several independent measurements, with a consequent accumulation of errors. In addition, the values for VI are probably consistently high because of the brownish color given in the Meijer test by some of the heterogeneous fractions. This color would mainly affect only compound VI because the latter is characterized by its yellow color in the Meijer test.

The calculations depend upon the assumption that the fluorescence and color tests given by each of the compounds are not affected by the others. The absence of large effects was established by preliminary tests, but detailed proof was impossible with the quantities of material available.

#### INSECTICIDAL TESTING METHODS

In making the insecticidal tests a representative sample of the coarsely ground beans was reground through the finest screen in the laboratory Raymond mill using 1 part of bean to 4 parts of Eastern Magnesia Talc (Emtco No. 23). This made a fine, rather oily dust which was used in all insecticidal tests. Since the dust was too oily to disperse well in a dusting tower, it was tapped out of a test tube through silk bolting cloth onto the larvae and leaves used in the test. Twenty-five third instar larvae of the Mexican bean beetle (*Epilachna varivestis* Muls.) were used in each test. Specimen dishes 110 mm. in diameter were lined with two thicknesses of moist paper toweling and a bean leaf was closely appressed to the bottom of the dish on the paper. The larvae were transferred to the test dish immediately before dusting. Mortality counts were made daily for 4 days. An insect was counted as alive if any movement was perceptible at the time of observation. No feeding occurred during the 4 days in any of the tests, and in nearly every case all insects were paralyzed so that the eventual kill with the concentrations and deposit used would be expected to be close to 100 percent. Four replicates were used in testing each material. Analysis of variance shows that highly significant differences exist between the samples tested.

#### RESULTS AND DISCUSSION

The source, species, chemical analysis, and toxicity to the Mexican bean beetle of each of the samples are presented in table 2.

#### CHEMICAL TESTS

The oil content of the different samples is strikingly constant. This fact is of interest because of the possible utilization of the oil as such, and because it eliminates the possibility of wide variations in physical or toxicological behavior of the different samples due to the effect of the oil.

The resin fraction, which contains practically all of the toxic components, shows considerable variation. The results of the color tests on the resin are roughly parallel to the quantities of resin, as might be expected.

The calculated quantities of the individual compounds also are roughly parallel to the resin content. The relative abundance of the compounds is approximately that expected from the quantities obtained in the work on isolation (7), with the exception of compound VI. This discrepancy may be accounted for both by the difficulty of isolating VI and by the interference in its colorimetric estimation already discussed under Sources of Error. The discrepancy is not serious, because of the lack of toxicity of VI. The somewhat erratic variation of compound I is not of great significance from the stand-

TABLE 2.—Origin, chemical analysis, and toxicity to the Mexican bean beetle of yam bean samples<sup>1</sup>

Sample	Origin	Altitude (meters)	Oil (percent)	Resin (percent)	Colorimetric values (percent rotenone equivalent)						Designated compounds calculated (percent)						Mortality in designated number of days (percent)													
					Meijer		Goodhue, 520 m $\mu$		Rogers- Calamari, 590 m $\mu$		I		II+V		III		IV		VI		VII		1		2		3		4	
					520 m $\mu$	430 m $\mu$	520 m $\mu$	430 m $\mu$	520 m $\mu$	430 m $\mu$	520 m $\mu$	430 m $\mu$	520 m $\mu$	430 m $\mu$	520 m $\mu$	430 m $\mu$	520 m $\mu$	430 m $\mu$	520 m $\mu$	430 m $\mu$	520 m $\mu$	430 m $\mu$	520 m $\mu$	430 m $\mu$	520 m $\mu$	430 m $\mu$				
C-43-17--	Yonkers, N. Y., greenhouse	250 (?)	25.7	1.5	0.60	0.90	0.26	0.50	0.08	0.24	0.10	0.21	0.28	0.07	4	6	33	37												
C-43-77--	Mexico, State of	275	1.6	0.85	1.35	1.63	.32	.71	.013	.25	.12	.24	.24	.04	.37	1	15	36	67											
C-43-82--	El Salvador, Department of Santa Ana	24.0	2.1	1.06	1.63																									
C-43-83--	do	75.0	24.0	1.8	1.96	1.52	.29	.65	.012	.36	.11	.22	.31	.34	.16	49	66	84												
C-43-84--	El Salvador, Department of San Salvador	26.3	2.4	1.22	1.86	.33		.77	.015	.44	.14	.24	.24	.54	.7															
C-43-88--	Peru, Iquitos	(?)	22.4	1.6	.56	1.14		.24	.022	.10	.09	.12	.22	.32	.2															
C-43-88--	Hawaii	(?)	28.4	2.8	1.37	2.13	.45	.84	.037	.47	.27	.32	.30	.40	.14	33	50	79												
C-43-90--	Mexico	(?)	25.3	1.9	.89	1.36	.35	.58	.020	.23	.15	.30	.38	.32	.4	31	49	71												
C-43-100--	do	(?)	26.5	2.1	.95	1.50	.36	.67	.024	.44	.18	.26	.24	.30	.22	12	22	36												
C-43-101--	El Salvador	(?)	24.5	2.2	1.04	1.63	.32	.69	.013	.43	.15	.22	.20	.30	.22	30	62	70												
C-43-104--	do	(?)	25.0	2.0	1.02	1.62	.30	.62	.009	.35	.11	.23	.23	.33	.41	14	48	67	82											
C-43-105--	El Salvador, Department of San Salvador	75.0	26.1	1.8	1.00	.30		.62	.011	.31	.11	.25	.33	.41	.14	27	40													
C-43-106--	El Salvador, Department of Santa Ana, Department of San Salvador	75.0	25.7	2.2	1.31	1.94	.39	.82	.011	.43	.14	.30	.30	.38	.2	32	56	84												
C-43-107--	El Salvador, Department of San Salvador	71.0	25.7	2.0	.90	1.38	.34	.67	.011	.43	.09	.21	.26	.26	.7	19	30	65												
C-43-108--	Mexico, State of Guerrero	71.0	24.5	1.8	.98	1.50	.36	.77	.008	.41	.05	.28	.35	.35	.25	41	60	73												
C-44-20--	Florida, Dade County	26.0	2.4	1.10	1.63	.45	.64	.015	.32	.11	.34	.33	.41	.44	.14	48	63	74												
C-44-22--	Guatemala, Department of Santa Rosa	22.5	2.4	1.00	1.97	.39	.61	.020	.35	.15	.22	.22	.42	.42	.19	29	44	55												
C-44-23--	Guatemala, Department of Santa Rosa	1,218	23.6	2.2	1.06	1.70	.32	.74	.015	.42	.10	.29	.30	.33	.3	18	35	39												
C-44-24--	Guatemala, Department of Zaltenapa	225+	23.3	3.4	2.14	2.93	.84	1.46	.004	.77	.07	.35	.31	.81	.27	61	80	84												
C-44-41 <sup>3</sup> --	Mexico, State of Chiapas	1,000±	19.6	2.6	1.07	1.70	.42	.92	.010	.50	.10	.32	.48	.48	.14	6	28	55												
C-44-42 <sup>3</sup> --	Hawaii	(?)	26.2	2.5	1.07	1.88	.39	.91	.020	.55	.20	.28	.63	.19	.6	28	50	72												
C-44-46--	Guatemala, Department of Cuitzapa	899	20.5	2.2	.95	1.46	.33	.84	.014	.51	.09	.25	.30	.30	.14	25	45	57												
C-44-47--	Guatemala, Department of Santa Rosa	1,218	21.3	3.3	2.02	2.90	.69	1.64	.006	.84	.43	.43	.43	.43	.12	.62	38	65	80											
C-44-86--	Mexico	(?)	21.0	1.8	.83	1.27	.29	.64	.022	.35	.15	.22	.21	.21	.25	.20	17	38	51											
6001a--	Mexico, State of Guanajuato	1,768	23.9	1.8	.70	1.13	.28	.49	.019	.21	.13	.18	.18	.18	.25	.18	35	44	73											
6001b--	do	(?)	24.4	1.8	.68	1.17	.27	.47	.020	.24	.12	.18	.18	.18	.22	.27	51	71												
6001c--	do	(?)	26.3	1.6	.82	1.26	.27	.63	.018	.36	.13	.20	.18	.22	.23	.15	55	62												
6002--	Mexico, State of Oaxaca	1,768	25.3	1.8	.78	1.25	.29	.58	.020	.29	.11	.25	.25	.25	.25	.21	6	22	39											
6003--	Mexico, State of Chiapas	1,526	26.2	1.8	.65	1.07	.25	.50	.021	.31	.13	.16	.20	.20	.22	.15	16	35	44											
C-44-158 <sup>4</sup>	Argentina, Buenos Aires	70.0	24.3	2.0	.95	1.52	.29	.65	.025	.36	.22	.20	.30	.30	.20	.12	20	29	48											

<sup>1</sup> Species *Pachyrhizus erosus* unless otherwise designated.<sup>2</sup> *P. tuberosus*.<sup>3</sup> *P. striatus*.<sup>4</sup> *P. ahupu*. Sample inadequate for chemical analysis.

point of insecticidal efficiency because this compound is nontoxic and has not yet been shown to have any relation to the other compounds. From the small quantity of compound V (erosone) originally isolated, and the relative abundance of II, it is very probable that the calculated values of  $\text{II} + \text{V}$  are made up largely of II. The values for IV (rotenone) are of great interest because of the toxicity of rotenone, and fortunately their determination is subject to relatively small errors by the present method. The values for VII (pachyrrhizid) are subject to considerable error, as already shown.

#### CORRELATION BETWEEN CHEMICAL AND INSECTICIDAL RESULTS

In the discussion of the chemistry of the yam bean (7) it was pointed out that of the various materials isolated, only rotenone and certain of the residues were toxic to the bean beetle. It was assumed, therefore, that a correlation might be found between the toxicity of different samples and the total resin or rotenone content or one of the colorimetric values. Any correlation with the quantities of the other compounds which might be found would be merely fortuitous. Correlation coefficients calculated between the toxicity and the analytical values are presented in table 3. The standard error of estimate in table 3 may be interpreted as the standard deviation of the number of bean beetle larvae killed in 4 days, as estimated from the chemical analyses.

The correlation coefficients are all significant, although not highly so. The standard error of estimate is high because of the many opportunities for experimental error in both the bio-assay and in the chemical analyses. The present results, however, establish as entirely practicable the use of one of the chemical determinations of table 3 to indicate those samples having a very high or a very low toxicity.

TABLE 3.—*Correlation coefficients and standard errors of estimate between 4-day mortalities of Mexican bean beetle larvae and analytical values of yam bean samples*

Analytical value	Correlation coefficient	Standard error of estimate
Resin fraction.....	0.376	15.9
Rotenone (IV).....	.409	15.6
Meijer color (520 m $\mu$ ).....	.420	15.6
Meijer color (430 m $\mu$ ).....	.395	15.7
Rogers-Calamari color.....	.391	15.8
Goodhue color.....	.387	15.8

The correlation coefficients for the several determinations are not sufficiently different to establish a clear-cut choice of the best method. It was previously shown (7), however, that the silkworm is very susceptible to compound V (erosone) and VII (pachyrrhizid) as well as to IV (rotenone). Other things being equal, a determination sensitive to all three of these compounds should give the best measure of toxicity to a variety of insects. Neither the Goodhue nor the Rogers-Calamari test is given strongly by all three compounds, while all three are sensitive to the Meijer test. Accordingly, the latter is suggested as the most satisfactory measure of the toxicity of yam bean samples,

based on the information available at present. Measurement at 520 m $\mu$  is less subject to interference from compounds II, III, and VI, and is therefore preferable to 430 m $\mu$ .

#### MORPHOLOGICAL AND ENVIRONMENTAL FACTORS

The data in table 2 suggest that no correlation exists between morphological variation of the yam beans, their toxicity, and their content of rotenone, but more tests and analyses are necessary before this evidence should be regarded as conclusive. Since only one sample each of *Pachyrrhizus strigosus*, *P. tuberosus*, and *P. ahipa* were tested, information for these species is scarcely comparable with that for *P. erosus*, the most variable species, of which 29 samples were tested. In view of the variability in toxicity and chemical composition of *P. erosus*, the normal expectation is that *P. strigosus*, *P. tuberosus*, and *P. ahipa* will also prove variable in these respects, but none of these species exhibits the morphological diversity of *P. erosus*. The seeds of *P. strigosus* are the smallest known for *Pachyrrhizus*. In insecticidal value, they compare favorably with the best samples of *P. erosus*. The seeds of *P. tuberosus* are the largest found in the genus, but the sample from Peru must be classed with the poorest ones of *P. erosus*. The samples of *P. erosus* include the major morphological variations of that species. Seeds of collection No. 6061 were graded on the basis of size and shape as follows: (a) large round seeds; (b) small round seeds; (c) large square seeds; and (d) small square seeds. In insecticidal value the round seeds in this series were slightly better than the square seeds and the larger seeds of each shape were somewhat better than the small seeds. In content of rotenone, however, the square seeds averaged higher. This sort of discrepancy is evident throughout the data in table 2. Although nothing is known of the genetical details, the tentative conclusion is that no real correlation exists between morphological characteristics and presence of rotenone. Structural characteristics and chemical composition in this instance seem to vary independently.

The best insecticidal samples of *Pachyrrhizus erosus*, based on combined chemical and toxicological data, support this view. Sample C-44-24 came from plants with lobed leaflets (var. *palmatilobus*). The seeds were small and mostly square. Sample C-44-47 included plants with both unlobed (var. *typicus*) and lobed (var. *palmatilobus*) leaflets, a condition frequently encountered in wild or cultivated stands of *P. erosus*. The seeds averaged slightly larger and were more rounded than those of C-44-24. The insecticidal properties of two species, *P. panamensis* and *P. vernalis*, have not yet been studied. Conceivably, data for these might alter some of the above statements, but these species are probably all closely related genetically and possibly are similar chemically.

Possible correlation between geographical occurrence or habitat and content of rotenone were also investigated. The data concerning origin in table 2 show that the samples of *Pachyrrhizus erosus* were obtained from diverse localities scattered throughout the general range of the species, as well as from different altitudinal levels. Consideration of these data fail to suggest either that plants from any particular

altitudinal level are better insecticidally or that those from any part of the range of the species are superior. Two of the best samples came from different sides of Guatemala, C-44-24 from the Department of Zacapa, at 225 meters, and C-44-47, from the Department of Santa Rosa, at 1,218 meters. Another sample, also from the Department of Santa Rosa, gave very poor results in tests with larvae of the Mexican bean beetle, and was only average in rotenone content.

### SUMMARY

The relationships between origin, chemical analyses, and insecticidal value of 31 yam bean samples have been studied in tests with Mexican bean beetle larvae. Significant correlations were found between toxicity and resin content, rotenone content, and three colorimetric analytical values. Neither the oil content nor six compounds other than rotenone isolated from the yam bean were significantly correlated with toxicity. The Meijer color test is proposed as a suitable chemical method for indicating approximately the toxicity of yam bean samples.

Morphological variation, geographical occurrence, and habitat have not been shown to affect the toxicity or composition of the sample. Three species of yam bean not previously studied, *Pachyrhizus tuberosus* (Lam.) Spreng., *P. strigosus* Clausen, and *P. ahipa* (Wedd.) Parodi, are reported as toxic to the larvae of the Mexican bean beetle.

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## PROGENY TESTS OF ASPARAGUS PLANTS<sup>1</sup>

By T. M. CURRENCE

*Associate horticulturist, Minnesota Agricultural Experiment Station*

### INTRODUCTION

The Washington strains of asparagus (*Asparagus officinalis* L.) were introduced by Norton (6)<sup>2</sup> as the F<sub>2</sub> progenies of individual plants. Presumably they were more or less heterozygous for vigor, and the original variability has probably not been reduced under the usual methods of seed propagation. Currence and Richardson (2) found that yields of individual plants on a uniform area of soil gave standard errors in percent of the mean of 79.5, 48.5, and 44.6 for the first, second, and third years of cutting. John Baer tomato plants, under somewhat similar conditions<sup>3</sup> have given a standard error of 25 percent of the mean for one year's yield. In the light of these facts it appears that inherited differences in vigor may exist between asparagus plants, and the important problem of identifying the better parental material and eliminating so far as possible the unproductive germ plasm faces the breeder. Superior breeding plants after being identified can be utilized in a number of ways to produce better seed than that from uncontrolled pollination. This paper presents the results of a study to obtain information regarding the efficiency of several methods of selecting and testing parental plants with some emphasis on the extent of improvement that may be obtained by these methods. The dioecious nature of the plants and the possibility of increasing individuals by asexual propagation make asparagus improvement problems somewhat unique among those of cultivated plants.

### MATERIAL AND METHODS

A planting of 1-year-old roots of a commercial strain of the Mary Washington variety was made in 1931, and harvesting records on single plants began in 1933. In 1937 open-pollinated seed was saved individually from a number of pistillate plants taken at random. Seeds of these were started in randomized block plantings in the greenhouse in the fall of 1937. In April of the following year the young seedlings were dug and a number of plants from each replicate were

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<sup>2</sup> Italic numbers in parentheses refer to Literature Cited, p. 76.

<sup>3</sup> Unpublished data.

weighed to obtain the comparative growth rates of the strains. Additional samples of the same strains were planted in the field at Grand Rapids, Minn. Facilities limited the size of the planting so that two plant plots were used with six replicates of each strain. Yield records were taken on this planting in 1940 and 1941. Also a few pistillate plants were crossed in pairs with a number of staminate ones. The pollinations were so designed that several combinations would be made for each of the parents. Progenies were started and transplanted in a test of six replicates in a balanced incomplete block arrangement. Harvests were made in 1941 and 1942. To compare the parent plants a test was started by dividing the crowns to form as many plants as possible. These clonal lines of parents were planted in randomized blocks, each clone being placed in as many blocks as the crown divisions permitted. The length of the harvesting period was approximately 6 weeks for each year. Spears were cut when they reached an estimated length of 6 inches or more. No trimming was done on the spears after harvest.

## RESULTS

### SELECTING FOR YIELD

The yields from the open-pollinated seed of individual plants are of special interest if the pollen may be assumed to have been a mixture from several staminate plants in the planting. The progenies from such seeds would be somewhat comparable to those of top crosses used in corn breeding as a general test for the combining ability of inbred lines. The use of open-pollinated seed may also be comparable to the polycross method suggested by Tysdal and Kiesselbach (10) for use with alfalfa, since the crosses are made without previous inbreeding. It may seem to be taking too much for granted to assume that the female plants were pollinated by pollen from several males rather than from only a few adjacent male plants. It has, however, been found that in a block of female plants consisting of three solid rows the center row consistently produces a crop of seed comparable to that produced on the outside rows which are adjacent to rows with a random distribution of male and female plants. In this instance, plenty of pollen has been transported across at least one row and this fact suggests that quite a mixture of pollen parents might occur when open-pollinated seed is saved from female plants growing in a random assortment of the two sexes. Therefore, the yields of the progenies are presented in table 1 as a comparison of the pistillate parents for breeding value and to identify different strains.

The data show significant differences among the progenies, which indicate that the female parents differed in their ability to produce high-yielding sexual progenies. The extent of the differences is apparent from the mean yields of the 19 progenies shown in table 1. Since the extreme between means is 0.98 ton per acre, with 0.385 being significant, it seems that advantage would be gained in commercial growing if seeds were obtained only from the better pistillate parents. The plants 3-5, 2-39, 5-11, 2-20, and 6-29 may be considered as 5 promising pistillate parents for breeding vigorous progenies. This type of test, if of sufficient extent might be helpful in identifying superior female parents for commercial production of seed or for

TABLE 1.—*Yield of pistillate asparagus plants and vigor of their open-pollinated progenies as young seedlings and as producing strains under field conditions*

Pistillate plant	4-year yield of pistillate plant	Mean seed- ling size of progenies	Calculated acre yields of progenies for 2 years
2-9	59.65	14.75	0.968
2-11	78.14	24.64	.971
2-20	71.02	24.72	1.206
2-35	23.95	33.75	.955
2-39	97.02	23.21	1.424
3-5	75.18	30.12	1.514
3-28	71.85	13.44	.678
3-34	25.28	22.50	.842
4-1	99.17	19.25	1.114
4-4	44.71	22.75	.748
4-15	64.40	19.25	.953
4-38	70.47	20.66	1.071
5-6	64.91	19.00	.536
5-11	54.00	13.69	1.256
5-41	54.10	21.00	.704
6-2	54.06	18.00	.867
6-6	84.12	19.00	.634
6-21	55.74	26.79	.838
6-29	47.51	20.17	1.226
Mean	62.91	-----	0.974
Significant difference	-----	-----	.385

further improvement work. If the 8 parent plants that were distinctly higher in yield than the mean of the 19 parents are considered as a group, it will be found that their progenies produced a crop approximately 10 percent larger than the average of all progenies. Thus, a 10-percent increase with this material would have been obtained by selecting these superior female phenotypes. It is also possible to obtain some information as to the effect of making a further selection at the time the progenies were in the seedling stage. If the 8 strains had been reduced to half that number on the basis of seedling size, the 4 remaining progenies with a seedling size greater than the mean of the 8 would be 2-11, 2-20, 2-39, and 3-5. One of these was only average in performance but the other 3 were superior, 2-39 and 3-5 being the 2 best-yielding lines in the planting. The yields of these 4 lines was 1,279, or an increase of approximately 31 percent over the mean of all strains. This would be a worth-while improvement. Yield tests on asparagus are expensive and difficult to make, so that a means of reducing the number of strains to be tested is of practical interest to the breeder.

The extent of improvement that would have been possible if an additional selection had been based on the comparative yields of the progenies and the lines had again been reduced to one-half should also be noted. Obviously 2-39 and 3-5 would have been chosen with a mean yield of 1.469 tons per acre, which is an increase of about 50 percent over the mean of 0.974 for all strains. From these results it is apparent that the essential points in such a selection program would be to save open-pollinated seeds from phenotypically superior pistillate plants and retain those that produce the largest seedlings. The field test would be the final progeny test and this would eliminate all except the best of the lines. It would then be possible to transplant and vegetatively increase the desired plants of the original ones selected. Such superior female plants if isolated with superior or even

average male plants should produce seed of greater productivity than the material from which it was selected.

Strains resulting from controlled pollinations of several different combinations of parental plants offer means of making a more satisfactory study of yield genotypes. From such pollinations definite combinations can be compared and information gained on the breeding value of both pistillate and staminate plants. The data in table 2 show yields and spear sizes of parent plants for the year 1942. The data are for one year on vegetatively propagated plants with various numbers of vegetative progeny from different plants. These were planted in randomized blocks in 1938 with the number of replicates shown as number of plants in test. There is wide variation within the vegetative lines which limits conclusive statements. The results on differences between the phenotypes of the parents are therefore considered as preliminary until further records are available. The standard errors were calculated as the variance within lines. The value of  $F$  for yields of pistillate plants was 2.47, the 5-percent point being 2.64. The value of  $F$  slightly exceeded the 5-percent point for spear size of pistillate parents. For the staminate parents the  $F$  value was not significant for either yield or spear size. The male parent plant which will appear later in the results as 3-9 was not represented with the asexual progenies because it was being used in other tests and could not be moved when the crowns were divided.

TABLE 2.—*Yield and spear size of vegetative lines of asparagus plants used in various combinations as parents of test progenies, 1942*

Pistillate plants				Staminate plants			
Identity of parent	Plants in Test	Yield per plant	Spear size	Identity of parent	Plants in test	Yield per plant	Spear size
	<i>Number</i>	<i>Ounce</i>	<i>Ounce</i>		<i>Number</i>	<i>Ounce</i>	<i>Ounce</i>
5-10-----	4	25.25±7.49	2.17±0.232	3-16-----	6	19.50±4.34	1.72±0.132
5-21-----	2	27.00±10.60	2.05±.328	2-12-----	6	20.83±4.34	1.58±.132
2-18-----	6	40.00±6.12	1.03±.189	5-32-----	2	13.00±7.52	1.40±.229
4-5-----	7	12.43±5.67	1.34±.175	4-17-----	9	27.11±3.54	1.42±.108
3-2-----	5	32.80±6.70	1.42±.207	5-2-----	4	14.75±5.32	1.65±.162
2-35-----	5	31.80±6.70	1.48±.207	2-3-----	2	10.00±7.52	1.70±.229

The progeny tests contained 31 progenies which were compared for yield, sex ratios, and spear size. Twenty-four strains composed the progeny from all possible combinations of 6 male plants and 4 female plants. Since this arrangement places parental plants of each sex on a comparable basis, the additional 7 lines are omitted from the yields shown in table 3. As previously stated, the strains were tested under field conditions for 2 years in a balanced incomplete block arrangement. The analysis of variance gives an  $F$  value of 4.0, which is definitely higher than the 1.85 required at the 1-percent point. It is obvious therefore that there were differences between the strains in the weight of asparagus produced by them during the 1941 and 1942 seasons. Table 3 shows the comparative yields on the basis of tons per acre with the 2 seasons' records combined. The means for progenies of each parent plant are shown in the last column of table 3 and the means of individual crosses in the body of the table. It is evident that the female plant 5-21 was superior to 5-10 and probably to 2-18,

with a *t* value of 1.89 for the latter comparison; 4-5 also was superior to 5-10 but was not significantly better than 2-18 or significantly poorer than 5-21. If it were desired to carry on one of these plants, it seems clear that the breeder would choose 5-21 as of most promise, since it is not inferior to the other three and is definitely superior to one of them.

TABLE 3.—*Yield<sup>1</sup> in tons per acre of asparagus progenies from various combinations of pistillate and staminate parents; grown in 6 replicates of 3 plants each*

Pistillate parent	Staminate parent						Mean <sup>2</sup>
	3-16	2-12	5-32	4-17	3-9	5-2	
5-10.....	3.650	3.902	2.612	3.778	2.901	3.742	3.431
5-21.....	4.433	4.035	3.734	4.143	4.828	4.732	4.318
2-18.....	4.493	3.884	3.790	3.954	4.366	3.408	3.989
4-5.....	3.879	4.251	3.334	3.631	4.470	5.446	4.169
Mean <sup>3</sup> .....	4.114	4.018	3.308	3.877	4.141	4.332	.....

<sup>1</sup> Significant difference=0.736.

<sup>2</sup> Significant difference=0.350.

<sup>3</sup> Significant difference=0.428.

Of the staminate plants 5-2 was slightly higher than any other with four crosses averaged. The differences are statistically significant only when 5-2 is compared with 5-32 and 4-17; 3-9 also exceeded 5-32 by a significant difference, but did not produce significantly higher yielding progenies than any of the other male parents.

It is possible that individual parent plants react quite differently in different combinations. The interaction, females  $\times$  males, when tested by the standard error, is significant in some instances. As an illustration of this the progenies from females 2-18 and 4-5 with the males 3-16 and 5-2 may be cited; 2-18  $\times$  3-16 is definitely a good progeny, but 4-5  $\times$  3-16 gives odds of about 15:1 that it is lower in yield. The combination 4-5  $\times$  5-2 was the highest yielding of any combination and 2-18  $\times$  5-2 was inferior in yield. The difference between the differences in tons per acre is  $2.551 \pm 0.612$ . Thus it is apparent that the pistillate plant 2-18 forms a favorable combination with 3-16 but a rather poor one with 5-2 and the pistillate plant 4-5 gives the opposite results with the two staminate plants. The results of these tests suggest that individual combinations of parental plants require consideration in breeding asparagus for increased production, and a method of using the best combinations for producing commercial seed would be valuable. With the exception of 5-32 all parent plants shown in table 3 were selected for superior yield. It is therefore impossible to make a completely satisfactory estimate of the degree of improvement that might be expected from seed of outstanding combinations. The highest yielding combination compared with the mean of 5-32 may provide a rough approximation. Inasmuch as 5-32 was an inferior phenotype and the females paired with it were superior ones the mean yield of the four combinations may not be greatly different from what would be expected from seed harvested from a commercial source. If this mean yield is compared with 4-5  $\times$  5-2 the difference is 2.078 tons per acre, or a possible increase of 61 percent due to the favorable combination.

The correlation coefficient between the two variables, mean yield of the two parents and their progeny yields, was 0.259 which does not approach the level of significance for the number of observations made. The parental yields were based on the data shown in table 2. The average of two parents was obtained by averaging the yield of the male and female parent of the particular cross. The two highest yielding parents in the test were 2-18 and 4-17 for the pistillate and staminate, respectively, but the progeny of this combination was not outstanding in yield. It was exceeded by a number of crosses and significantly so by a few. Furthermore, one of the best yielding progenies in the test was that of 4-5 × 5-2. Both of these parents would be considered low in yield as indicated by table 2, but in the production of high-yielding progenies they rank high. Because of the high standard errors of the parental clones, the information obtained from these crosses is not entirely satisfactory, but it does suggest that there may not be sufficient correlation between the vigor of parents and the yield of progenies to give special emphasis to the former in selecting for greater yield. The interaction between genes brought together seems to be of most significance, although, generally speaking, vigorous selections may have better combining ability than weaker ones. Since an adequate number of high-yielding parent plants will normally be available for breeding work, it would seem advisable to use only the most productive for progeny testing and by the progeny test to determine which of them are desired for further use.

#### RATIO OF PISTILLATE TO STAMINATE PLANTS

Robbins and Jones (8, 9) have demonstrated that male plants are more productive than female. Since a disproportionate ratio of the sexes might therefore affect the comparative yields of the strains, it is of interest to compare the various progenies for the number of male plants in each. Table 4 shows the numbers of the two sexes produced by the various pairs of parents. The data on sex ratios were recorded after all plants had flowered. In addition to those in the

TABLE 4.—*Sex ratios of 31 asparagus progenies from the indicated combinations of parental plants*

Pistillate parent	Sex of progeny	Staminate parent							Open-pollinated	Total
		3-16	2-12	5-32	4-17	3-9	5-2	2-3		
5-10-----	Male-----	12	12	15	13	14	8	9	-----	83
	Female-----	10	14	9	11	9	16	9		
5-21-----	Male-----	14	8	12	8	17	13	-----	72	
	Female-----	10	12	11	14	16	10	-----		
2-18-----	Male-----	15	12	19	9	14	13	-----	82	
	Female-----	16	12	20	11	8	10	-----		
4-5-----	Male-----	10	8	12	11	14	17	-----	72	
	Female-----	14	14	12	13	10	17	-----		
3-2-----	Male-----	-----	8	7	-----	12	-----	-----	27	
	Female-----	-----	10	10	-----	5	-----	-----		
2-35-----	Male-----	-----	8	-----	-----	9	-----	-----	17	
	Female-----	-----	10	-----	-----	8	-----	-----		
L-43-----	Male-----	-----	-----	-----	-----	-----	-----	9	9	
	Female-----	-----	-----	-----	-----	-----	7	-----		
Total-----	Male-----	51	40	74	48	59	72	9	9	362
	Female-----	40	42	72	59	233	56	9	7	318

<sup>1</sup> *P* less than 0.05.

<sup>2</sup> *P* less than 0.01.

test planting, some extras were grown in guard rows. In four individual progenies the deviation from the 1:1 ratio was great enough to be significant by the  $\chi^2$  test, suggesting a trend toward excess male plants. However, there are a few of the progenies having excess females which also approach significance.

Totals for parent plants show that only one of them 3-9, had progenies deviating significantly from the theoretical ratio. This also is an excess of male plants and probably in numbers sufficient to indicate that strains with excess male plants are possible as an asparagus-breeding objective. Such strains would have the advantage of containing fewer of the less productive female plants. The data on total progenies such as that of 5-10, 2-18, and 5-2 where the deviations are large but do not give 20 to 1 odds are considered as inconclusive. An additional planting of open-pollinated seed from 2-18 was grown. Combined with the data shown in table 4, the totals are 77 female: 88 male plants with a  $P$  value only slightly greater than 0.5. The information available suggests that 3-9 is the only plant tested which can be considered as producing with some consistency an excess of staminate plants in the progeny. Other progenies now being grown from 3-9 contain 40 female: 69 male. The total numbers therefore are 73 female: 128 male, a highly significant deviation from the 1:1 ratio.

Rick and Hanna (*7, p. 712*) suggest "partial sex linkage of some lethal factor" as a genetic explanation of excess male plants in certain strains. Since their data showed that sex in asparagus is controlled by a single gene, occasional crossover types would be expected between the sex gene and the partially linked lethal. In the event of crossing over, there should occur elimination of the male sex. Also it would be essential for the lethal gene to be incompletely dominant since a completely dominant lethal immediately eliminates itself from a population. To explain the data on progenies of 3-9 an incompletely dominant lethal linked with the recessive female-producing gene might be postulated. Incomplete or partial dominance would account for the parent being in existence since this allows for some survival. At the same time, such dominance would account for the uniform elimination of a part of one sex or the other which seems to occur in the progenies of 3-9. It is also necessary to postulate that all the female parents were normal or recessive for the lethal gene and that approximately 33 percent crossing over occurred between the male gene and the lethal. Thus, 67 percent of the female-producing gametes would be eliminated and 33 percent of the male ones. This would tend to give the approximate ratio observed if some few male gametes with the lethal gene survive to give slightly more than one-third males.

Although the numbers are too small to furnish conclusive results, some interesting trends are suggested by crosses other than those involving the 3-9 parent. Among these are the three combinations or crosses that gave significant deviations from the 1:1 ratio, all excess plants being males. None of the deviations for excess females reached significance though a few approached it. Because of the nature of the combinations that gave the significant deviations from the 1:1 ratio, it is suggested that the interaction of genes from the

two parents may effect the elimination or survival of one or both of the sexes and so bring about the disproportionate sex ratios observed.

Because of the possibility that some of the yield differences shown in tables 1 and 3 might have resulted from variations in the sex ratios of the different crosses, correlations between number of male plants and yield between and within the 31 strains were calculated. Within strains there was a very low positive relation, but between strains the coefficient of correlation was only 0.096. From this it is thought that the factors which affected the yield of the strains tested were generally independent of the sex ratios.

#### SPEAR SIZE

Producers of asparagus generally prefer spears of large diameter; hence the possibilities in breeding for uniformly large spears are of interest. In 1942 a large number of spears were taken from each plot of the balanced incomplete block planting. The diameter of each spear was measured at the thickest part and the diameter means for the plots were calculated. These means have been used to compare the spear sizes of the various progenies, to compare the diameters with the weights of spears, and to test the relation of the spear size of parents with that of their progenies by means of covariance.

The spear size of available parent plants based on average weight in ounces are shown in table 2. The female plants differed significantly as judged by the *F* value; the *F* value for the male plants was not statistically significant.

TABLE 5.—*Spear diameter of strains of asparagus in centimeters and the average spear diameter of certain pairs of parents*

Pistillate parent	Generation	Staminate parent							Mixture	Mean of progenies and parents
		3-16	2-12	5-32	4-17	3-9	5-2	2-3		
5-10.....	Progeny.....	1.39	1.46	1.17	1.21	1.18	1.24	1.10	-----	1.25
	Parents.....	1.95	1.88	1.79	1.80	-----	1.91	-----		1.86
5-21.....	Progeny.....	1.29	1.67	1.33	1.43	1.28	1.28	-----	-----	1.38
	Parents.....	1.88	1.82	1.72	1.74	-----	1.85	-----		1.80
2-18.....	Progeny.....	1.21	1.14	1.03	1.11	1.19	1.08	-----	-----	1.13
	Parents.....	1.37	1.30	1.22	1.23	-----	1.34	-----		1.29
4-5.....	Progeny.....	1.12	1.22	1.11	1.23	1.29	1.07	-----	-----	1.17
	Parents.....	1.53	1.46	1.37	1.38	-----	1.50	-----		1.45
3-2.....	Progeny.....	-----	-----	1.10	1.30	-----	1.05	-----	-----	1.15
	do.....	-----	-----	1.04	-----	-----	1.05	-----		1.05
L-43.....	do.....	-----	-----	-----	-----	-----	-----	-----	1.15	1.15
	Mean.....	1.25	1.37	1.13	1.26	1.24	1.13	1.10		-----
	Progeny.....	1.68	1.62	1.53	1.54	-----	1.65	-----		

Among the progenies some produced the large diameter usually desired. The data in table 5, in which spear size of progenies is compared with that of the average of the parents, suggest that this character may be determined by the phenotype of the parent. The cross which gave the greatest spear diameter was 5-21 × 2-12, with a mean of 1.67 cm. The mean of the parents was 1.82 cm., somewhat near the maximum spear size for pairs of parents. The cross giving the smallest spears was 2-18 × 5-32 with a mean of 1.03 cm. This cross was from parents of small size. Although several exceptions must be noted, the data seem to offer some possibilities of selection for spear size based on spear size of parent plants. Inasmuch as the parents were partly se-

lected for large stalks it is probable that practically all these progenies have larger than average spears. Two exceptions are the progenies of L-43 and those of the staminate parent 5-32. The latter was a small, weak plant and the former progeny was produced by a mixture of pollen, so that progenies of these come from crosses in which only one parent was superior and therefore they would be expected to exceed the average to a less extent than progenies having both parents selected. Thus it may be assumed that any population in this test exceeding a spear diameter of about 1.10 cm. would be greater than average commercial stock in size of spears produced.

The covariance analysis for testing the data for correlation between spear weight and spear diameter is shown in table 6. Since it is less arduous to count spears and weigh them in bulk than to measure diameter, it is of interest that the correlation coefficient gives a high value, showing that diameter may be predicted fairly accurately from spear weight.

From the standpoint of general desirability the cross 4-5 × 3-9 appears to be one of the best. It gave a fairly good yield with satisfactory size of spears. Other crosses produced larger spears and some were more productive but it is doubtful whether any were superior in both respects.

TABLE 6.—*Analysis of covariance for spear weight and diameter of 31 strains of asparagus*

Source of variation	Degrees of freedom	Sum of squares or product			Mean square or product				Regression of diameter on weight
		Diameter	Products	Weight	Diameter	Products	Weight	r	
Blocks....	30	2.258	1.745	2.203	0.075	0.058	0.073	0.782	0.792
Progenies....	30	3.123	3.239	4.220	.104	.108	.141	.892	.767
Error....	125	6.255	1.130	2.403	.050	.009	.019	.292	.470
Total....	185	11.636	6.114	8.826	.063	.033	.018	.460	.693

#### DISCUSSION

In an asparagus improvement program the genetic combinations brought together are no doubt important, and indiscriminate isolating of productive plants for seed production does not appear very promising as a means of obtaining superior seed. For male parents it would seem desirable to have progeny tests of several combinations. If in doing this a male plant is found that produces uniformly vigorous progenies, it might be vegetatively divided and grown in a seed plot. Progeny testing of female plants is somewhat easier than that of male plants if use is made of open-pollinated seed. This seed, of course, should be obtained from a planting in which pollen from several different males was utilized. Female plants chosen by this method should then be put in an isolation plot with males that have demonstrated good combining ability. In the planting of such a plot advantage should be taken of the fact that the plants can be vegetatively reproduced. Several more female plants will be needed than males; 1 male plant to about 10 or 12 female plants should be sufficient to provide the necessary pollen. With a rather crude technique a 4-year-old crown has been divided into 20 plants, and an even greater num-

ber might be obtained with proper technique. If such divisions are repeated at intervals, the eventual building up of a fairly adequate supply of parent plants should not prove too difficult.

Tysdal and Kiesselbach (10) have called attention to the possibility of using vegetative propagation and self-sterility to obtain commercial seed of alfalfa. This plan provides for the use of the best single cross known and obviates the necessity of inbreeding several generations. Since asparagus is dioecious it would seem feasible to employ somewhat the same method, although the supply of seed produced would be rather small for a good many years. An isolation plot might contain only 1 male clone with 10 or 12 females if they are known to combine well with the staminate plant. Seed from the different female clones would then be single crosses and might be marketed separately as individual single crosses if desired. Starting with 1 staminate and 5 pistillate parents and dividing them every 4 years into 10 plants each, would require 3 divisions or 8 years to build up to 6,000 plants, or roughly the number required to plant an acre at the common spacing of 1.5×5 feet. Since in 12 years 10 acres could be planted, this plan seems rather promising. Manifestly in starting with only 2 parents, 1 staminate and 1 pistillate, the program would be considerably delayed by getting the female clone built up. Because of this about 12 years might be needed to obtain 2 acres of seed-producing plants.

A rather serious limiting factor in this program might be the labor needed to dig and divide a large number of old asparagus crowns, but it is also reasonable to expect that methods could be developed that would reduce the labor required. If resources were adequate the divisions might be grown in a loose soil in containers and then removed by washing. This would preserve the root system and speed up the program. No doubt other time-saving methods would be evolved by workers if this type of improvement were applied.

Prior to 1942 a small beginning was made on increasing promising clones. A few plants of 3-9 and 5-2 are now growing in isolated plots with a few plants each of 4-5 and 2-18. There is no assurance that repeated propagation by crown divisions will be successful, but no serious difficulty has been experienced in getting vigorous plants from the first division.

To produce a large amount of commercial seed, the possibilities of a double cross from outstanding parents should be investigated. In corn-breeding work Jenkins (5) and others have demonstrated the importance of making double crosses from the proper single crosses. With four inbred lines of corn there are six possible single crosses and three possible double crosses, but since asparagus is dioecious there are only four possible combinations from four plants. In corn the methods of predicting the performance of double crosses from the performance of single crosses are shown by the work of Jenkins (5), Anderson (1), Doxtator and Johnson (3), Hayes and Johnson (4), and others.

It appears quite accurate to estimate double-cross yields from the average of four single crosses that contain neither of the combinations entering into the parentage of the double cross. From the results given in table 3 it would seem that the double cross (5-21 × 5-2) ×

(4-5 × 3-9) should be a good one. These are the most promising parents from the standpoint of breeding behavior for yield as well as for other characters and the single crosses were the two lower yielding of the four possible combinations. It would therefore be expected that this double cross would give a more vigorous population and greater yield than the double cross from the more productive single crosses. By building up clonal lines of the original parents and isolating them in proper combinations, seed of single crosses could be obtained. This seed would then be planted and the two sexes identified. Staminate plants from the one single-cross parent would be put in an isolated planting with pistillate plants from the other single cross. In a second isolation plot the opposite sexes from the two crosses would be grown. Seed produced from these isolated plots would be double crosses and should exhibit considerable hybrid vigor although not as much as the single crosses. Within a few years a large amount of commercial double-cross seed could readily be produced by this method.

Rick and Hanna (7) suggest another approach to the problem of getting productive strains. They outline a plan for breeding an all-male population to take advantage of the greater production by male plants and also to provide the seed producer with monopolistic control of his product. This would seem a very promising line of attack. Although the writer has been unable to find viable seed on male plants, Rick and Hanna imply that there would be no particular difficulty in doing this under the conditions of their experiments. With homozygous male plants available and information on their combining ability as well as on that of the female parents, it should be possible to obtain a very productive strain.

A small beginning toward inbreeding has been made by sib-crossing some of the better strains from known parents and one of foreign origin. It is hoped after several generations of sib-crossing to have inbred strains that, when combined, will show sufficient hybrid vigor to provide a productive single cross for commercial purposes. Considering the favorable results that this method has given with other crops, especially corn, it should be adequately tested for asparagus. The nature of the plant and its perennial habit offer some serious obstacles but also certain advantages. If suitable strains are once obtained and identified for proper combinations it will be comparatively simple to maintain the lines and combine them as desired for commercial seed production. A fact which may have considerable significance in the choice of one or several of the possible methods of asparagus improvement is that by retaining all parental material, the originator would be in a position to maintain a monopoly on the commercial seed and should be able to realize a profit in proportion to the degree of improvement that the material offers over other strains.

#### SUMMARY

Open-pollinated seed from 19 pistillate asparagus plants was grown and records were taken on seedling size and yields of the 19 strains. The better phenotypes tended to produce better progenies than the poorer ones so that a yield increase of about 10 percent might be expected from the best 8 of the 19 parent plants. If a further selection

had been made at the time of transplanting an increase of about 30 percent would have been obtained. The desirability of making progeny tests was shown by the 2 best lines producing a 50-percent greater crop than the mean of all lines.

Progenies from different combinations of parental plants of both sexes were compared for yield, sex ratio, and spear size. Distinct differences were noted between parents for transmitting yield genes and between single crosses for favorable combinations. It is thought that the combination of dominant yield genes of the parents needs emphasis in the development of high-yielding strains of asparagus as it does for corn and other crops. Yields of parent plants, vegetatively divided, did not seem to bear any outstanding relation to the yields of their progenies either in several combinations or in individual combinations.

Total progenies from one male parent differed significantly from the theoretical sex ratio. All significant deviations were the result of excess males with the disproportionate ratios approaching 1 female : 2 males. However, the number of males in these progenies appeared to exert little effect on the yields of single crosses. Because of a few combinations which gave deviations approaching significance and those which were significant, the possibility is suggested that interaction of genes might eventually be found to be the genetic basis for disproportionate sex ratios.

Average spear size of the paired parents seemed to be a rather poor indication of the spear sizes to be expected in the progenies. By measuring diameters and weighing samples from the different plots it was found that diameter was highly correlated with weight so that weight provides an adequate estimate of spear diameter.

Several methods of breeding asparagus for more productive strains are discussed.

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# THE QUANTITATIVE ESTIMATION OF HEMICELLULOSES BY DIRECT ISOLATION<sup>1</sup>

By H. D. WEIHE, *assistant chemist*, and MAX PHILLIPS, *senior chemist, Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, United States Department of Agriculture*

## INTRODUCTION

The various methods described in the literature for the quantitative estimation of hemicelluloses were reviewed in an earlier paper (5)<sup>2</sup>. It was pointed out that the analytical methods, based either on the determination of the quantity of reducing sugars produced when these carbohydrate complexes are subjected to acid hydrolysis or on the determination of the percentage of furfural, afford results that are difficult to evaluate in terms of actual quantity of hemicellulose in the material under examination.

Preece (6) has introduced a method for the quantitative estimation of hemicelluloses based on the isolation and weighing of the hemicelluloses. According to the Preece procedure, the sample (20-25 gm.) is given a preliminary extraction first with a hot 0.5-percent aqueous ammonium oxalate solution, then with a 1-percent boiling sodium hydroxide solution in 50-percent ethanol, and finally with a 50-percent ethanol solution. The residual material is then repeatedly extracted, at room temperature (25°-30° C.), with a 4-percent aqueous sodium hydroxide solution, until a portion of the extract no longer gives a precipitate when neutralized with acetic acid and diluted with an equal volume of acetone. The combined alkaline extract is then rendered acid by the addition first of glacial acetic acid and then of acetone equal in volume to the acidified solution. The precipitate is filtered, dried in vacuo and the weight of the moisture- and ash-free product is determined. The result thus obtained is calculated as percentage of free hemicellulose. The material remaining from the extraction with the alkali solution is further extracted with 4-percent boiling aqueous sodium hydroxide solution, and the product is isolated as previously described. The result is reported as percentage of combined hemicellulose.

So far as the writers are aware, no attempt has yet been made to examine the Preece method critically. In a slightly modified form, the method was used by Buston (2, 3) and by Bennett (1) in their studies of the cell-wall constituents of plants. However, when the plant material is extracted according to the Preece procedure, not only are the hemicelluloses removed but some of the lignin also, with the result that both the free and combined hemicellulose fractions of Preece are contaminated with lignin. Moreover, when the extraction

<sup>1</sup> Received for publication July 11, 1945.

<sup>2</sup> Italic numbers in parentheses refer to Literature Cited, p. 85.

of combined hemicellulose is made with 4-percent aqueous boiling sodium hydroxide solution, considerable degradation of the hemicelluloses is likely to occur.

In the present paper, a procedure is described for the quantitative estimation of hemicelluloses in straw, which consists essentially of two steps: (1) the isolation of the holocellulose from a sample which has previously been freed of alcohol-benzene extractives and pectic substances, and (2) the isolation of the hemicellulose from the holocellulose. The residual material is weighed to determine the total amount of material extractable with dilute sodium hydroxide solution. This method for the quantitative estimation of hemicelluloses is believed to have definite advantages over the other methods described in the literature. It is realized, however, that no method entirely free from criticism can be developed with our present limited knowledge of the chemistry of hemicelluloses.

#### METHODS OF EXPERIMENTATION

##### APPARATUS

The apparatus shown in figure 1 was found to be very useful in determining the percentages of pectic substances and holocellulose. It consists of a fritted-glass crucible, *B*, 5½ inches tall and 1¼ inches in diameter, the lower end of which was rimmed to fit the rubber stopper

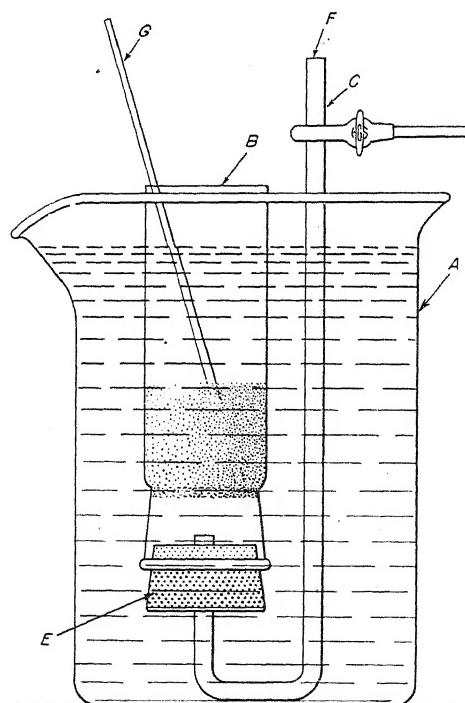


FIGURE 1.—Apparatus for determining pectic substances and holocellulose.

*E.* The fritted-glass disk of the crucible was made from ground glass, 80-100 mesh. Through an opening in the stopper *E* the tube *C* is passed and any solvent in *B* can be removed by applying suction at *F*. The contents of *B* are mixed with the glass rod *G*. With this apparatus it was possible to make the two determinations with the minimum manipulation and without the necessity of transferring the sample from one container to another.

#### EXTRACTION OF THE SAMPLE

The determination of the 1:2 alcohol-benzene extractives in the plant material under investigation was carried out as follows: A 5-gm. sample was placed in the crucible *B* which was then inserted into a Soxhlet extraction apparatus and the extraction was allowed to proceed for 30 hours. Then the crucible was taken out, and as much as possible of the adhering solvent was removed by suction. The crucible and its contents, next dried in vacuo at 60° C., were finally weighed.

The extraction of pectic substances (and other constituents soluble in the 0.5-percent aqueous ammonium oxalate solution) was carried out as follows: To the crucible *B*, the rubber stopper *E* and the tube *C* were attached. The crucible was nearly filled with hot (85° C.) 0.5-percent aqueous ammonium oxalate solution and the entire assembly was placed in beaker *A* which was filled with water and maintained at a temperature of 85°. The crucible was allowed to remain in the beaker for 1 to 1½ hours, while the plant material and ammonium oxalate solution were stirred from time to time with the glass rod *G*. After each extraction period, the solution in *B* was removed by applying suction at *F* and the digestion with fresh 0.5-percent ammonium oxalate solution was then repeated. After the removal of all pectic substances (determined by adding to a portion of the ammonium oxalate extract four times its volume of ethanol, previously acidified with hydrochloric acid, and then noting whether any precipitate separated out), the residual plant material was washed with water until free of ammonium oxalate, then washed with 95-percent ethanol, dried in vacuo at 60°, and weighed.

The holocellulose in the plant material, which had been successively extracted with alcohol-benzene and ammonium oxalate solutions, was next determined, a modification of the procedure recommended by Ritter and his coworkers (4, 7) being followed. A somewhat different procedure was used for the chlorination of the sample, and the washing with cold water after chlorination was eliminated.

The chlorination was carried out in a special apparatus, illustrated in figure 2, consisting of a glass jar *B* provided with a glass cover *D*, which had been ground to fit tightly to the top of *B*. Through the opening in *D*, the one-holed rubber stopper *O* was inserted. The glass tube passing through the stopper *O* was connected by means of a rubber tube provided with a Hofmann clamp, *E*, to the T tube *P*. *P* in turn was connected by means of suitable tubes, as shown in the drawing, to the bottles *H* and *J* and to the one-liter suction flask *N* containing dilute sodium hydroxide solution for the absorption of chlorine. The various rubber tubes were provided with the clamps *F*, *G*, *I*, *K*, *L*, and *M*. *H* was almost filled with a saturated solution of sodium chloride. Chlorine from a tank entered the system at *Q*. By closing

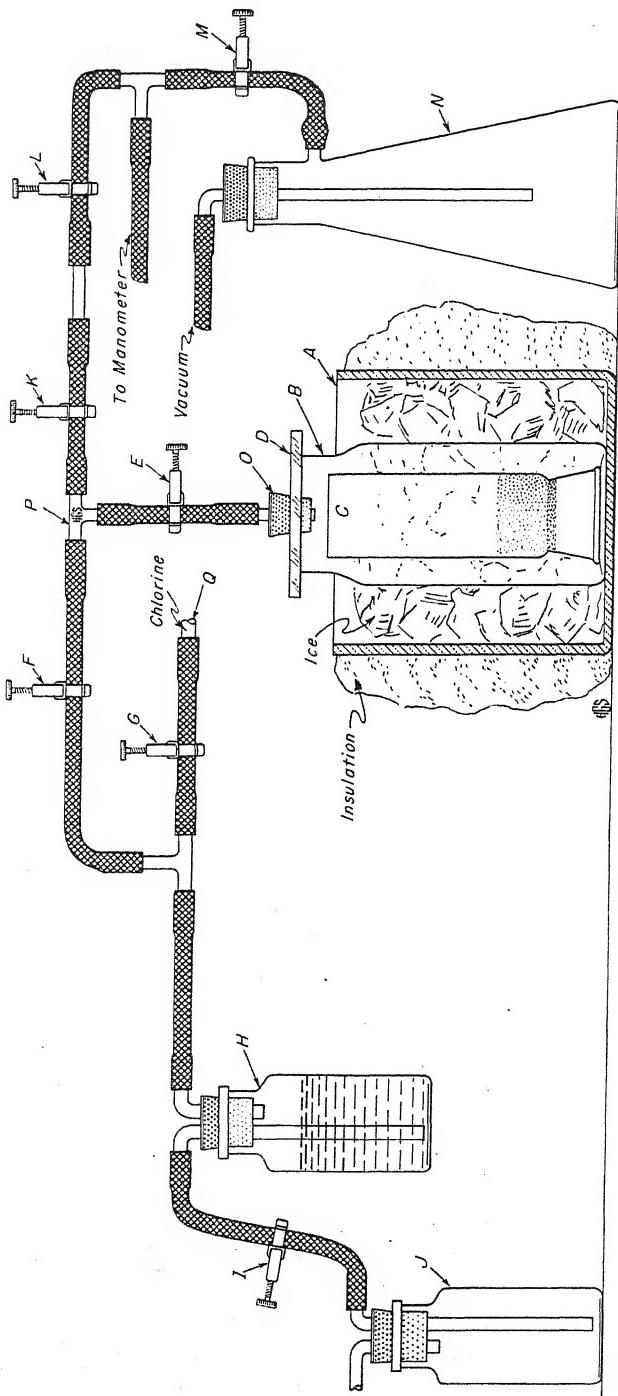


FIGURE 2.—Chlorination apparatus.

the clamp *F*, and opening the clamps *G* and *I*, chlorine from *Q* could be led into *H* and the salt solution driven over into *J*. *B* was placed in an insulated jar *A* and the space between *A* and *B* was filled with crushed ice. The sample to be chlorinated was contained in the fritted-glass crucible *C*.

#### ISOLATION OF HOLOCCELLULOSE FROM THE SAMPLE

The determination of the holocellulose was carried out as follows: The plant material in figure 2, *C*, which had been successively extracted with 1:2 alcohol-benzene solution and with 0.5-percent ammonium oxalate solution was moistened with about 12 ml. of distilled water and placed in the chlorination chamber *B*. *B* was placed in *A* and the latter was filled with crushed ice. *D* was placed on the top of *B* and *O* was replaced with a thermometer provided with a suitable stopper. The thermometer was inserted to extend within an inch of the sample in *C*. When the temperature in *C* was 15° C., or below, the thermometer was removed and *O* was inserted into the opening of *D*. With *F* closed and *E*, *K*, and *L* open, the system was evacuated by opening *M*.

By means of a monometer not shown in the drawing, it was determined whether the system was gastight before the chlorine was permitted to enter. With *K*, *L*, and *M* closed and *G* and *I* open, chlorine from a tank was first passed through a gas wash bottle containing water and was then allowed to enter at *Q* until about one-half of the salt solution in *H* was displaced and forced into *J*. *F* was slowly opened, while the flow of chlorine entering at *Q* was increased. The valve on the chlorine tank and *F* were so regulated that the level of the liquid in *H* remained about the same. When the pressure of the chlorine in *B* was approximately atmospheric, *H* was allowed to fill with chlorine. Next the valve of the chlorine tank and the clamp *G* were closed. Then the level of the liquid in *H* rose slowly, owing to the absorption of the chlorine by the sample in *C*. When the absorption of chlorine had ceased, *F* was closed. *C* was removed from the chlorinating chamber *B*; 95-percent ethanol was added to *C*; and the sample and the ethanol were thoroughly mixed with a glass rod. The ethanol was removed with suction and the washing with this solvent was repeated. To *C* was then added a 3-percent ethanolamine solution in 95-percent ethanol and the contents were stirred with the glass rod. After it had stood for 2 minutes, the solution in *C* was drawn off with the aid of a suction flask. The treatment with the ethanolamine solution was repeated. The reaction product was washed twice with ethanol; once with ether; and was then freed of solvent with the aid of the suction pump.

The sample in the crucible *C* was moistened again with about 12 ml. of distilled water. Then the chlorination and the subsequent treatments with ethanol, ethanolamine solution, ethanol, and ether were repeated. This process of alternate chlorination and extraction was repeated until the chlorinated sample no longer produced a coloration with ethanolamine solution. The crucible *C* and its contents were dried in vacuo at 60° C., weighed, and the percentage of holocellulose in the sample was calculated.

## ISOLATION OF HEMICELLULOSES FROM HOLOCCELLULOSE

The experiments hereinafter described were undertaken for the purpose of determining the optimum conditions for extracting the hemicelluloses from holocellulose. The following procedure unless specifically indicated otherwise, was used in these experiments:

To 1 gm. of holocellulose, contained in a 200-ml. Erlenmeyer flask, a specified volume of 4-percent aqueous sodium hydroxide solution was added, the flask was stoppered, and the mixture was allowed to digest at room temperature ( $25^{\circ}$ - $30^{\circ}$  C.) for a specified period. From time to time the reaction mixture was shaken manually. At the end of the allotted interval, the reaction mixture was filtered on a cloth through a small Hirsch funnel; the residual material was washed with 15 ml. of water; and the washings were added to the main alkaline filtrate. To the filtrate 3 volumes of ethanol were added, the solution was made slightly acid with acetic acid, and the mixture was allowed to stand overnight at room temperature. The supernatant liquid was then drawn off, and the hemicellulose material was filtered in a weighed sintered glass crucible of fine porosity and washed with 100 ml. of an aqueous alcoholic solution (made by adding 25 ml. of water and 1 ml. of glacial acetic acid to 75 ml. of 95-percent ethanol). It was next washed successively with 95-percent ethanol, absolute ethanol, and ether; then dried in vacuo at  $60^{\circ}$  and weighed.

An experiment was first carried out for the purpose of determining the rate of extraction of the hemicelluloses from holocellulose. To 1 gm. of holocellulose, 100 ml. of a 4-percent aqueous sodium hydroxide solution was added. After the reaction mixture had been allowed to stand at room temperature for a given period, it was filtered, and the hemicelluloses were isolated as described above. The residual material resulting from the extraction of the hemicelluloses with the alkaline solution was subjected to successive extractions, at room temperature, with aqueous 4-percent sodium hydroxide solution. The hemicelluloses obtained in each extraction were determined separately.

TABLE 1.—*Successive extraction of hemicelluloses from 1 gm. of wheat straw holocellulose, at room temperature, during a 2-hour period, with 100 ml. of 4-percent aqueous sodium hydroxide solution*

Order of extraction	Weight of hemicelluloses Gram	Weight of residue Gram	Loss or gain	
			Percent	
First.....	0.3227			
Second.....	.0128			
Third.....	.0066			
Fourth.....	.0034			
Fifth.....		0.5770		-7.75
Sixth <sup>1</sup> .....	.0486	.4862		-4.22
Seventh <sup>1</sup> .....	.0418	.4454		+.10

<sup>1</sup> The extraction of the residual material was made at  $100^{\circ}$  C. during a period of 1 hour.

These operations were continued until a portion of the alkaline extract, on the addition of 3 volumes of ethanol and acidulation with acetic acid, gave no precipitate. The residual material after being washed with alcohol and ether and dried was weighed and then extracted twice at  $100^{\circ}$  C. with a 4-percent aqueous sodium hydroxide solution, each extraction lasting 1 hour. The results obtained are

recorded in table 1. (All results on the yield of hemicelluloses represent the average of at least two determinations.)

From the results shown in table 1 it can be seen that even in the alkaline extract from the third successive extraction of wheat straw holocellulose, some hemicellulose equivalent to nearly 0.7 percent of the starting material was obtained. It appears, then, that the removal of the hemicelluloses from wheat straw holocellulose, at room temperature, proceeds rather slowly, since four successive extractions were required to remove all the readily available hemicelluloses. The subsequent extraction of the residual material at 100° C. resulted apparently in a progressive degradation of the cellulose.

In the hope that the time required for separating the hemicelluloses from the holocellulose might be reduced, a series of extraction experiments at room temperature was carried out under the same conditions as those recorded in table 1, except that the extraction period was reduced to 1 hour. The results obtained are recorded in table 2.

TABLE 2.—*Successive extraction of hemicelluloses from 1 gm. of wheat straw holocellulose, at room temperature, during a period of 1 hour, with 100 ml. of 4-percent aqueous sodium hydroxide solution*

Order of extraction	Weight of hemicelluloses	Weight of residue	Loss	
			Gram	Percent
First.....	0.3192			
Second.....	.0099			
Third.....	.0075	0.5869		7.65
Fourth.....	0			

It will be observed from a comparison of tables 1 and 2 that during the 1-hour extraction period the total yield of hemicelluloses (0.3366 gm.) was somewhat less than that obtained during the 2-hour period (0.3455 gm.). The loss, or difference between the starting material and the sum of the weights of the hemicelluloses and residue, was about the same in both series of experiments.

In a third series of experiments the holocellulose was exhaustively extracted, first with a 2-percent aqueous sodium hydroxide solution; and then with a 4-percent aqueous sodium hydroxide solution. It was believed that the weaker alkali solution might afford a greater yield of hemicellulose, presumably because of its less drastic action on the hemicelluloses. The results obtained are recorded in table 3.

TABLE 3.—*Successive extraction of hemicelluloses from 1 gm. of wheat straw holocellulose, at room temperature, during a 2-hour period, first with 100 ml. of 2-percent aqueous sodium hydroxide solution and then with 100 ml. of 4-percent solution*

Order of extraction	Concentration of sodium hydroxide solution	Weight of hemicelluloses	Weight of residue	Loss or gain	
				Percent	
First.....	2	0.2852			
Second.....	2	.0024			
Third.....	2	0	0.6442	-6.82	
First.....	4	.0444			
Second.....	4	0	.6070	+.72	

It will be observed from table 3 that the total amount of hemicelluloses extracted by the 2-percent sodium hydroxide solution was 0.2876 gm. The total extracted, by both concentrations of the solution, was 0.3320 gm. The extraction of the holocellulose first with 2-percent and then with 4-percent aqueous sodium hydroxide solution gave a lower yield of hemicelluloses than the 4-percent extraction shown in table 1, although the loss sustained when the extraction was carried out with 2-percent aqueous sodium hydroxide solution (table 3) was somewhat less than in the experiments recorded in table 1. The small apparent gain obtained when the residual material, which had already been extracted with 2-percent aqueous sodium hydroxide solution, was subjected to extraction with 4-percent aqueous sodium hydroxide solution was undoubtedly due to an experimental error.

Next a series of experiments was carried out which differed from the previous series only in that the extraction was made first with a 1-percent instead of a 2-percent aqueous sodium hydroxide solution. The hemicellulose fraction extracted by the 1-percent solution amounted to 0.2086 gm., whereas that extracted by the 4-percent aqueous sodium hydroxide solution was 0.1197 gm. The total yield of hemicelluloses was 0.3283 gm., somewhat less than the yield obtained in the experiments recorded in table 3. The loss resulting from the extraction operations was approximately the same as that sustained in the previous series of experiments. There appeared to be no advantage in making the extraction with the 1-percent aqueous sodium hydroxide solution.

It may be of interest in this connection to mention the fact that when 1 gm. of wheat straw holocellulose was digested for 2 hours with 100 ml. of distilled water, 0.1128 gm. of hemicellulose was obtained. The loss amounted to 4.28 percent.

#### DISCUSSION

When the technique described above is followed, no difficulty is experienced in getting consistent checks of the percentage of holocellulose in the sample. It is, of course, conceivable that, even under the relatively mild conditions employed for the isolation of holocellulose, some group or fraction constituting an integral part of the hemicellulose complex may have been split off. However, it is believed that any error arising from this source cannot be appreciable.

The development of a satisfactory method for the quantitative estimation of hemicelluloses in holocellulose is complicated by the fact that the hemicelluloses are a rather ill-defined group of carbohydrate complexes. For the purpose of this investigation, hemicelluloses have been defined as carbohydrate complexes that are extracted from the holocellulose with dilute aqueous sodium hydroxide solution, at room temperature, and that are precipitated from the alkaline extract by the addition of an excess of ethanol. While this definition may be arbitrary to some extent, this would be equally true of any other definition of hemicelluloses that might be proposed.

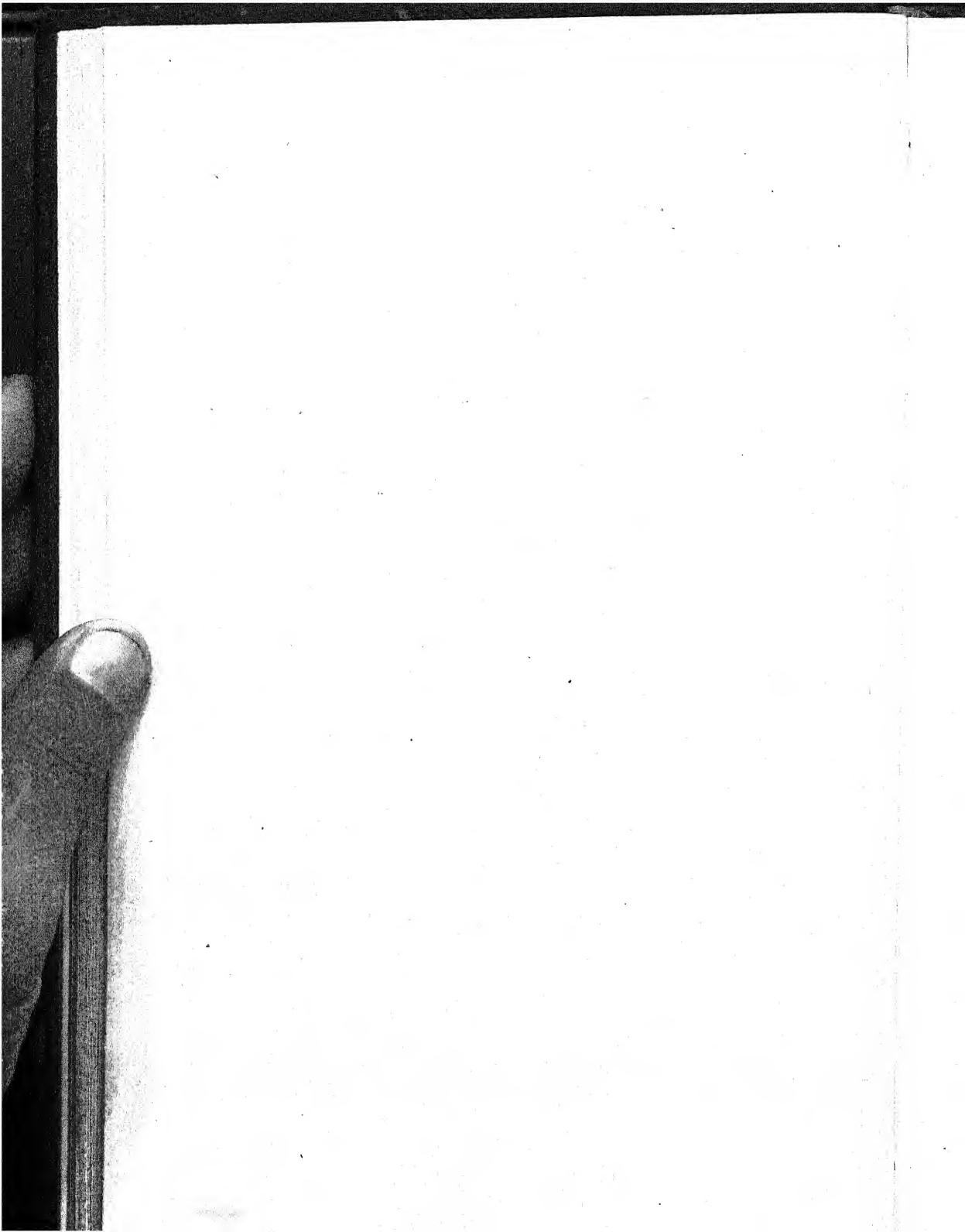
## SUMMARY

A method for the quantitative estimation of hemicelluloses in wheat straw is described. It involves first the isolation of holocellulose from a sample which had previously been extracted with a 1:2 alcohol-benzene solution and with a 0.5-percent aqueous ammonium oxalate solution, and then the removal of the hemicelluloses from the holocellulose by extraction, at room temperature, with a 4-percent aqueous sodium hydroxide solution.

The maximum yield of hemicelluloses is obtained when the holocellulose is extracted at room temperature four successive times, for periods of 2 hours each, with a 4-percent aqueous sodium hydroxide solution.

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# SOME CHEMICAL TREATMENTS AND THEIR INFLUENCE ON DAMPING-OFF, WEED CONTROL, AND WINTER INJURY OF RED PINE SEEDLINGS<sup>1</sup>

By A. J. RIKER, professor, R. H. GRUENHAGEN, L. F. ROTH, formerly research assistants, Department of Plant Pathology, Wisconsin Agricultural Experiment Station, and W. H. BRENER, Manager, Griffith State Nursery.<sup>2</sup>

## INTRODUCTION

The control studies described herein were made because damping-off continues to influence production in Wisconsin forest nurseries. The causal fungi seem to be distributed widely in cultivated soil, but to be relatively scarce in unbroken forest soil (?).<sup>3</sup> While serious outbreaks are sporadic, small losses are expected each year. The unpredictable occurrence of epidemic attacks in the various nurseries is a handicap to the sustained production demanded by an extensive planting program.

In Wisconsin damping-off is caused principally by two fungi, *Pythium irregularare* Buisman and *Rhizoctonia solani* Kühn, although seedlings are occasionally damped-off by other micro-organisms. These two fungi induce damping-off with somewhat different symptoms and are active under different circumstances, so that control measures against one may have little effect on the other.

For example, *Pythium* operates in Wisconsin at relatively cool temperature, high humidity, and in neutral to slightly acid soil. In contrast, *Rhizoctonia* is active particularly in relatively warm, dry soil with an acid reaction (8, 9). Nevertheless, control measures need to be effective against both, and they are not especially differentiated in this paper.

Reasonable commercial control has often been secured in Wisconsin by planting on newly cleared forest land, by fall seeding, by covering late spring seeding with sand, and by soil treatments, especially with dilute sulfuric acid. These methods have been used alone or in various combinations. Nurserymen used new land as long as the nurseries were expanding, but, eventually, they reached a limit when they had to replant land that had already grown tree seedlings. Fall seeding has been the more common practice, but sometimes circumstances have required spring planting. Late spring planting

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<sup>3</sup> Italic numbers in parentheses refer to Literature Cited, p. 95.

with the seed covered by sand is a nursery application of the greenhouse practice common with many kinds of susceptible plants.<sup>4</sup>

Both the sterilizing effect of sulfuric acid and the increased soil acidity prevent damping-off and kill many weed seeds. However, acid treatment is relatively expensive to apply and is harmful to the nurseryman's clothes and skin if improperly handled. There is some possibility of chemical injury to the seedlings, but worst of all (10) is the fact that the acid undesirably disturbs the base exchange capacity of the soil.

This paper presents studies aimed at a control of damping-off as efficient as sulfuric acid but at one that will not change conspicuously the nutritional balance in the soil. An abstract covering some of the work has already appeared (6).

#### MATERIALS AND METHODS

In the early stages of the work, many different trials were made in 4- and 6-inch pots in the greenhouse where a number of unpromising materials were eliminated.

The soil employed was Plainfield sand secured from near the location of nursery trials at Wisconsin Rapids in order that the greenhouse studies might be more directly applicable to nursery conditions in central Wisconsin. An analysis of this soil is available (8).

Seed from local red pine (*Pinus resinosa* Ait.) was used for the most part, but some observations were also made with seed from Scotch pine (*P. sylvestris* L.) and from Austrian pine (*P. nigra* Arn.).

The fungi used for soil inoculation were *Pythium* and *Rhizoctonia*, as described by Roth and Riker (8). Greenhouse pots were planted with a counted number of seeds, usually 25, 50, or 100 seeds per pot, depending primarily upon its size. The different treatments were made with suitable numbers of replications. The soil in some of the trials was steamed for 20 minutes at 15 pounds pressure in 6-inch pots. However, most of the trials had a natural woods soil practically free (8) from damping-off fungi.

For those treatments employing seed dusted with a disinfectant, the seed and dust were placed in a drum about 4 inches in diameter and 7 inches long which was provided with 1-inch baffles. The drum was closed and slowly rolled for 10 feet. The seed was removed from the drum, separated from excess dust over a fine mesh screen, and placed in packets prior to planting.

For nursery plantings one could easily treat the seed in grain dusters, such as those in common use (4).

The nursery trials were made in three Wisconsin locations. Nurseries 1 and 2 were located on Plainfield sand in central Wisconsin, while No. 3 was on Vilas light sandy loam in northern Wisconsin. Both of these soils had a pH near 5.7, and for some years previous had been employed in growing nursery pine trees. The seed and fungi were similar to those in the greenhouse studies. Where the soil was inoculated, damping-off fungi were introduced from sand-corn-meal cultures grown in mason jars. The fungus and its corn-meal substrate were passed through a quarter-inch wire mesh and worked

<sup>4</sup> The Evergreen Nursery, Sturgeon Bay, Wis., has eliminated its damping-off problem by covering the seed with Lake Michigan sand.

into the surface soil of the seedbed at the rate of 1 pint of culture for 16 square feet.

Dry soil treatments that were strong enough to injure the seed were applied evenly over the surface of the soil, were worked in to a depth of 1.5 inches, and were allowed to stand 3 days before seeding. Seeding was made without disturbing more than this top 1.5 inches of soil, and the calomel treatment was made at this time. The chemical was dusted into the seed trenches at the rate of 0.4 gm. per square foot of plot area.

All of the liquid treatments were applied just after seeding, at the rate of one-half pint per square foot. The liquid-treated beds were then leached with an equal volume of clear water.

All of the seedbeds were covered with wire screen and half shade to prevent injury by rodents, birds, and excessive sunshine. In both the smaller and larger field plantings the various treatments in each replication were distributed by chance.

Records of emergence and damping-off of the small-scale plantings were made at frequent intervals. Records on germination included the entire area planted, but those for damping-off excluded 3 inches marked off around the edge to eliminate marginal effects.

Seedlings which were damped-off and those which were chemically injured were differentiated according to the descriptions of Hartley (2, 3) and Roth and Riker (7). All standing seedlings were given a slight brushing pull upward. If they came up, they were counted according to the character of the lesion. If they resisted the pull, they were considered healthy. This gave the benefit of the doubt to seedlings with minor chemical injury.

## EXPERIMENTAL RESULTS

### GREENHOUSE STUDIES

The following chemicals were employed in the greenhouse studies in liquid soil treatments: Sulfuric acid, sodium dinitro-ortho-cresolate, mercuric chloride, ferrous sulfate, copper oxide, copper chloride, and aluminum sulfate. In dry soil or seed treatments the following were used: Mercuric phenyl cyanamide and cadmium oxide, mild mercurous chloride, ethyl mercury iodide, paraformaldehyde, cuprous oxide, cuprous chloride, and cuprous carbonate, ethyl mercury phosphate, finely divided metallic mercury in talc, ammonium phosphate, tetramethyl thiuramdisulfide, hydroxymercurichlorophenol, tetrachloroparabenoquinone, sodium salt of 2-, 4-, 5-trichlorophenol, and pentachlorophenol.

Various plant materials were used also to determine whether they might either contain antibiotics against the damping-off fungi or stimulate the growth of micro-organisms antagonistic to these pathogens. These materials included ground oak leaves, pine needles, alfalfa, grass, autoclaved oats, sawdust, and corn meal. Since none were effective, further mention of them is omitted.

Nine different sets of greenhouse trials were made between 1937 and 1945 in order to determine which materials were sufficiently promising for nursery testing. Because of their volume, they are not described in detail.

From these greenhouse trials several materials appeared encourag-

ing as used, and worthy of nursery trials. The most promising were a seed dust of mercuric phenyl cyanamide and cadmium oxide and more particularly a soil application of calomel. The combination of these treatments indicated a control of damping-off approximately as good as that from sulfuric acid. However, this acid apparently killed many weeds which were not affected by these mercurials.

The possible advantages of these or similar chemicals were as follows: (1) They had no accident hazard such as that provided by pouring water into concentrated sulfuric acid. (2) They were less injurious to the skin or clothing on contact than dilute sulfuric acid. (At the same time it is clear that these chemicals are poisonous if eaten, that the dust should not be inhaled in any quantity, and that continual skin contact might be harmful.) (3) They were easier to handle. (4) They had no obviously ill effect on the fertility of the soil. Since traces of mercury had disappeared within a few months (5), there was no fear that the minute amount employed per square foot might accumulate.

The more promising of these substances along with certain other commonly used chemicals were employed in experimental tree nursery trials.

#### SMALL SCALE NURSERY TRIALS

Of the chemicals tried in the greenhouse, all were employed in small nursery beds except cuprous oxide, cuprous chloride, cuprous carbonate, and metallic mercury.

Small-bed nursery trials were made during the period 1937 to 1943, inclusive. There were two trials in nursery No. 1, seven in nursery No. 2, and four in nursery No. 3. The individually treated areas were either 2 by 2 or 3 by 4 feet in size. Each trial consisted of a number of treatments (3 to 22) and replications (4 to 6) as appeared necessary. Although most of the details of these studies have been omitted because of their volume, some of the results of a trial on inoculated soil are given in table 1, as representative of the group.

The percentages of damping-off for each of the six replicates in the three critical treatments shown in table 1 were as follows: No treatment—18, 7, 45, 11, 10, and 42; sulfuric acid—2, 8, 3, 12, 7, and 30; combination—4, 1, 3, 3, 2, and 1. No statistical analysis seemed necessary to conclude that in this trial the combination was significantly superior to no treatment and as effective as the sulfuric acid

TABLE 1.—*Averages from 6 replications of small-scale nursery trials on the control of damping-off of red pine seedlings, made in 1939, on inoculated soil at nursery No. 1*<sup>1</sup>

Treatment	Seed planted that emerged	Emerged seedlings that were—		
		Damped-off	Chemically injured	Healthy
None	Percent	Percent	Percent	Percent
Sulfuric acid <sup>2</sup>	70	22	0	78
Combination <sup>3</sup>	66	10	2	89
Seed dust alone	83	2	0	98
Soil dust alone	80	15	0	85
	84	7	0	93

<sup>1</sup> 11 of the less promising among 16 treatments have been omitted.

<sup>2</sup> 2 percent sulfuric acid, 3 gallons per 4 by 12 foot bed (235 ml. per square foot).

<sup>3</sup> Seed dust of mercuric phenyl cyanamide, 8 percent, and cadmium oxide, 2.5 percent (commercial Barak C); soil dust treatment of calomel, 0.4 gm. per square foot.

against damping-off. Particularly in the sulfuric acid plots, some of the seedlings classed as damped-off may have been chemically injured.

Isolations were made from damped-off seedlings in various treatments. *Pythium* was rather less prevalent than *Rhizoctonia* (ratio 15 to 21) in the untreated plots and was a poor second to it (7 to 22) in the sulfuric acid plots. However, *Pythium* appeared exclusively (16 to 0) in the plots treated with calomel.

The combination treatment appeared sufficiently promising to justify trials on a commercial scale.

#### COMMERCIAL SCALE TRIALS

Commercial nursery trials were made with the best of the treatments from the 9 greenhouse and 13 small-bed nursery trials, viz, (1) sulfuric acid, (2) mercuric phenyl cyanamide with cadmium oxide seed dust, (3) calomel on the soil, and (4) a combination of (2) and (3).

The trials consisting of five treatments and six replications were made in 1940, 1941, and 1942. The field manipulations were similar to those described for the small-bed trials. The results with controls, sulfuric acid, and the seed and soil treatments alone and combined (table 2) show some of the variability encountered. The combina-

TABLE 2.—Averages from 6 replications of large-scale nursery trials on the control of damping-off of pine seedlings

Location, year, and pine seed	Treatment	Seed planted that emerged	Emerged seedlings that were—		
			Damped-off	Chemically injured	Healthy
Nursery No. 1:		Percent	Percent	Percent	Percent
1940 Austrian	(None)	27	16	0	84
	Sulfuric acid <sup>1</sup>	32	2	4	94
	Combination <sup>2</sup>	28	6	0	94
	Seed dust alone	28	7	0	93
	Soil dust alone	28	7	0	93
1941 Red	(None)	79	12	0	88
	Sulfuric acid	76	5	0	95
	Combination	74	1	0	99
	Seed dust alone	71	10	0	90
	Soil dust alone	77	2	0	98
1942 Scotch	(None)	18	30	0	70
	Sulfuric acid	16	11	2	87
	Combination	20	9	0	91
	Seed dust alone	19	25	2	73
	Soil dust alone	20	8	1	91
Nursery No. 2:		Percent	Percent	Percent	Percent
1940 Red <sup>3</sup>	(None)	84	2	0	98
	Sulfuric acid	75	3	15	82
	Combination	80	1	0	99
1941 Red <sup>3</sup>	(None)	81	8	0	92
	Sulfuric acid	78	2	7	91
	Combination	81	2	0	98
1942 Red <sup>3</sup>	(None)	87	2	0	98
	Sulfuric acid	87	1	1	98
	Combination	86	1	0	99
Nursery No. 3:		Percent	Percent	Percent	Percent
1940 Red	(None)	83	4	0	96
	Sulfuric acid	83	1	1	98
	Combination	85	1	0	99
	Seed dust alone	81	3	0	97
	Soil dust alone	85	1	0	99
1941 Red	(None)	76	25	0	75
	Sulfuric acid	74	13	2	85
	Combination	70	19	0	81
	Seed dust alone	71	41	1	58
	Soil dust alone	73	30	1	69

<sup>1</sup> 2 percent sulfuric acid, 3 gallons per 4 by 12 foot bed (235 ml. per square foot).

<sup>2</sup> Seed dust of mercuric phenyl cyanamide, 8 percent, and cadmium oxide, 2.5 percent (commercial Barak C); soil dust treatment of calomel, 0.4 gm. per square foot.

<sup>3</sup> Results from seed dust alone and soil dust alone are omitted, because there was too little damping-off to indicate effectiveness.

tion treatment, and frequently the calomel soil dust alone, caused less chemical injury to seedlings than sulfuric acid and was as effective in controlling damping-off. On the other hand, sulfuric acid eliminated many weeds that were not affected by the other treatment.

#### REDUCTION IN NUMBER OF WEEDS

Because weeds often present a critical problem in the seedbed, an effort was made to find a chemical that would destroy many weed seeds, and control damping-off, without causing either injury to the pines or undesirable changes in the soil.

The following materials were tested: In liquid soil treatments, sulfuric acid and sodium dinitro-ortho-cresolate; in dry soil treatments, tetramethyl thiuramdisulfide, ethyl mercury iodide, ethyl mercury phosphate, tetra-chloro-parabenoquinone, sodium salt of 2-, 4-, 5-trichlorophenol, mercurous chloride, and hydroxymercurichlorophenol.

These materials were employed in four trials of from three to six replications each in the greenhouse in 1941, 1942, and 1943, and in four trials each with four small-bed replications in two forest nurseries. Although several of the materials gave fair weed control, all but tetramethyl thiuramdisulfide, as used, failed to meet other requirements. A summary of portions of the field trials is given in table 3. For brevity the treatments with little promise have been omitted.

TABLE 3.—*Averages from 4 replications of soil treatments against weed seeds and damping-off in red pine seedbeds*

Soil treatment	Measure used	Amount per square foot	Weeds per 12 square feet	Seed planted that emerged	Emerged seedlings that were—		
					Damped-off	Injured by chemicals	Healthy
1942 Nursery No. 2:				Number	Percent	Percent	Percent
None				102	85	6	94
Calomel <sup>1</sup>	Grams	0.4	55	83	3	2	95
TMTD, pure <sup>2</sup>	do	.3	57	86	1	1	98
Do	do	.6	24	85	2	1	97
Do	do	1.2	9	86	1	2	97
Sulfuric acid, 2 percent	Milliliters	236	17	77	2	5	93
1942 Nursery No. 3:				Number	Percent	Percent	Percent
None				105	72	2	98
Calomel <sup>1</sup>	Grams	.4	85	73	2	0	98
TMTD, pure <sup>2</sup>	do	.3	86	64	1	1	98
Do	do	.6	24	71	1	0	99
Do	do	1.2	10	64	1	1	98
Sulfuric acid, 2 percent	Milliliters	236	5	80	0	1	99
1943 Nursery No. 2:				Number	Percent	Percent	Percent
None				448	89	8	92
Calomel <sup>1</sup>	Grams	.4	264	90	5	0	95
TMTD, 50 percent <sup>2</sup>	do	1.2	107	86	12	0	88
Do	do	2.4	58	86	7	0	93
Do	do	4.8	17	95	4	0	96
Sulfuric acid, 2 percent	Milliliters	236	63	86	3	25	0
1944 Nursery No. 2:				Number	Percent	Percent	Percent
None				70	76	13	87
TMTD, 50 percent <sup>2</sup>	Grams	4.8	10	61	0	0	100
Sulfuric acid, 2 percent	Milliliters	236	28	81	2	0	98

<sup>1</sup> Seed also treated with mercuric phenyl cyanamide and cadmium oxide (commercial Barbak C).

<sup>2</sup> Tetramethyl thiuramdisulfide (pure was commercial Tuads, 50 percent was commercial Thiosan).

<sup>3</sup> This increase in the amount of damping-off following acid treatment has never been encountered in any other trials. The readings for the 4 replications, respectively, were as follows: 23, 27, 25, and 25 percent. Similar increases appeared with 2 other chemical treatments. *Rhizoctonia* was particularly severe in 1943.

The amount of damping-off in these trials, especially in 1942, was not large enough to give much information about damping-off control in the nursery. However, in these trials tetramethyl thiuramdisulfide appeared equal to sulfuric acid in respect to (1) weed control, (2) control of damping-off, (3) chemical injury to seedlings, and (4) seedling emergence. The significance of the weed control was apparent from inspection.

One of these experiments (1943, nursery No. 2) was in progress during a season when winter injury was severe.

#### WINTER INJURY

The winter of 1943-44 was particularly severe on pine seedlings in several Wisconsin forest nurseries. There was no rain, sleet, or snow during December. The lack of adequate snow cover caused a large amount of frost heaving and winter drought. However, in the spring of 1944, some plots that had been treated and seeded in 1943 survived the winter better than others.

The treatments and seedings were made in April 1943 with three replications. The results (table 4) were significant by inspection, so no statistical analyses were made. The data indicate that the use of increasingly larger amounts of the chemical gave concomitantly improved protection against winterkilling. The results presented are on only 1 year's observations in one locality. Since such winters are unusual, the opportunities are limited for repeating such observations. The writers hope they will be tried in regions where such winter injury is common. At the same time the influence of hydrophilic colloids in the soil (1) deserves consideration.

TABLE 4.—*Summary of survival of red pine after various soil treatments in relation both to damping-off and to winter injury*

Soil treatment	Year	Measure used	Amount per square foot	Seedlings from seed planted
None:				Percent
Emergence, June and July.....	1943	.....	.....	88
Seedlings living, August.....	1943	.....	.....	81
Seedlings living, July.....	1944	.....	.....	33
Sulfuric acid (2 percent):				
Emergence, June and July.....	1943	milliliters.....	235	86
Seedlings living, August.....	1943	.....do.....	235	64
Seedlings living, July.....	1944	.....do.....	235	20
Calomel 1:				
Emergence, June and July.....	1943	grams.....	4	90
Seedlings living, August.....	1943	.....do.....	4	85
Seedlings living, July.....	1944	.....do.....	4	33
Tetramethyl thiuramdisulfide:				
Emergence, June and July.....	1943	grams.....	1.2	86
Seedlings living, August.....	1943	.....do.....	1.2	76
Seedlings living, July.....	1944	.....do.....	1.2	63
Tetramethyl thiuram disulfide:				
Emergence, June and July.....	1943	grams.....	2.4	86
Seedlings living, August.....	1943	.....do.....	2.4	80
Seedlings living, July.....	1944	.....do.....	2.4	71
Tetramethyl thiuramdisulfide:				
Emergence, June and July.....	1943	grams.....	4.8	95
Seedlings living, August.....	1943	.....do.....	4.8	92
Seedlings living, July.....	1944	.....do.....	4.8	83

<sup>1</sup> Seed also treated before planting with mecuric phenyl cyanamide and cadmium oxide.

## DISCUSSION

The variability inherent in the damping-off problem has indicated that any control measure may give fluctuating results from place to place in the same year and from year to year in the same place. In Wisconsin, the activity of the two principal fungi changed with different circumstances. For example, *Pythium* ordinarily was active in cool, wet, and slightly acid to neutral soil, while *Rhizoctonia* appeared in warm, moderately dry, and relatively acid soil (7, 8, 9). The Plainfield sand is light enough to absorb rain easily and to blow when not protected. In another locality and with a different kind of soil, one might expect these or different pathogenic fungi to operate in various ways—and correspondingly that other control measures might be necessary.

An increase in damping-off has appeared from time to time when the chemical treatment was quite mild. Examples appear in table 2 (nursery No. 3, 1941 trials), in the 1943 trials of table 3, and have been encountered frequently among chemical treatments not recorded because they were not effective against damping-off. Of the factors that might influence this increase, two deserve special mention.

Chemical injury and damping-off are difficult enough to distinguish when they are operating separately. As Roth and Riker (?) have pointed out, seedlings that have been weakened may be more susceptible to damping-off than vigorous seedlings. As a result, under certain conditions a chemical injury too mild to be classed as such may open the way for damping-off fungi and thus provide more damage than no treatment at all.

A more complicated consideration involves antagonisms between different biological agents in the soil. Damping-off fungi have to compete against other kinds of soil fauna and flora, including *Trichoderma*. Perhaps some of the mild chemicals injure or destroy the inhibitors of the damping-off fungi and, thus, indirectly favor the development of damping-off.

The effectiveness of tetramethyl thiuram disulfide is difficult to explain. Possibly its well-known antioxidational properties would particularly affect the *Rhizoctonia*. Further study is suggested on its repellent or destructive effect on soil fauna. For example, if it inhibited worms that attacked roots, and flagellates that killed nitrifying bacteria, the seedlings would have improved water-absorbing systems and a better nutritive balance to withstand winter injury. If further trials were made with this chemical, the related but less expensive commercial "Thiuram M" might be included.

While the authors have achieved their purpose in finding chemicals, in addition to sulfuric acid, that control damping-off and that do not obviously upset the nutrient balance in the soil, the question of relative costs is quite pertinent. These chemicals cost more than sulfuric acid. However, they need less labor to apply, and they require no supplementary application of fertilizer to restore the desirable base exchange capacity disturbed by the acid. Counting everything, the cost of using these chemicals seems close enough to that of sulfuric acid to deserve consideration.

In any case, it seems better, when possible, to avoid damping-off by such cultural means as may best suit the circumstances; e. g., (1) the use of newly cleared land, (2) fall seeding, or (3) late spring seeding with the seed covered by sand rather than soil.

### SUMMARY

A search has been made for a means of controlling damping-off in Wisconsin forest nursery seedlings which would be as effective as dilute sulfuric acid, but which would not obviously upset the nutritional balance in the soil.

A seed treatment of mercuric phenyl cyanamide with cadmium oxide, supplemented by a soil treatment with calomel, was relatively effective against damping-off from *Pythium* and *Rhizoctonia* in Wisconsin's sandy soil.

A soil treatment with tetramethyl thiuram disulfide in limited trials was relatively effective not only against this damping-off but also against many weed seeds and winter injury.

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# SOME PHYSIOLOGICAL CHARACTERISTICS OF FOUR VARIETIES OF SPRING WHEAT PRESUMABLY DIFFERING IN DROUGHT RESISTANCE<sup>1</sup>

By ARTHUR T. BARTEL<sup>2</sup>

Assistant agronomist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture

## INTRODUCTION

Varieties of wheat, *Triticum aestivum* L. (*T. vulgare* Vill.),<sup>3</sup> differ in drought resistance, as indicated by their yields of grain and their growth characteristics under semiarid or drought conditions. To determine to what extent the supposed differences in drought resistance of Baart, Ceres, Marquis, and Hope wheats are correlated with the moisture content of the leaf blades and with the osmotic pressure and total solids of the extracted leaf-blade juices, the growth characteristics of these four varieties were studied at Tucson, Ariz., in irrigated and nonirrigated (drought) plots, 1934 to 1938.

## REVIEW OF LITERATURE

Drought resistance, according to Maximov (10, p. 399),<sup>4</sup> is the "capacity of plants to endure drought and to recover readily after permanent wilting, with the minimum of damage to the plant itself

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<sup>3</sup> *Triticum aestivum*, originally applied by Linnaeus to the bearded spring wheats only, under the International Rules of Botanical Nomenclature becomes the specific name for all common bread wheats, since it was the earliest name applied to any of the varieties of this group. The concept of this species as emended by Host under the name *T. vulgare* Vill. is that now most generally accepted by wheat specialists. Wheat specialists likewise have preferred the name *T. vulgare* because it is more truly descriptive of the species. The name *T. vulgare*, however, is untenable under present rules of nomenclature, since it was originally proposed by Villars merely to replace the earlier name, *T. aestivum*. It is hoped, however, that at an appropriate time the rules can be amended so that the name *T. vulgare* can be conserved for this species.

<sup>4</sup> Italic numbers in parentheses refer to Literature Cited, p. 111.

and to the yield produced." Briggs and Shantz (2) stated that permanent wilting in plants occurs when the moisture content of the soil in which they are growing is reduced to the point where the plants will not recover when placed in a water-saturated atmosphere. They will, however, slowly recover after the addition of water to the soil. Such wilting is injurious to the plants (10), especially to the embryonic tissue of the stem and to the root hairs.

The rates of water loss of cut plants of Baart and Ceres wheat were found by Bayles, Taylor, and Bartel (1) to be less than those of Marquis and Hope. Martin (9) reported that the rate of water loss in sorghum leaves is less than in corn leaves. On the other hand, Newton and Martin (14) found that timothy and western ryegrass had about the same rate of water loss, even though the latter is the more drought-resistant.

Miller (12) reported that the leaf moisture of corn was higher than in the drought-resistant crops kafir and milo. No relations were found by Flerov, Brokert, and Levin (4) between the water content of spring wheat varieties and their drought resistance. Schopmeyer (15) found that the drought-resistant shortleaf pine had a higher leaf-moisture content and a lower osmotic pressure than the nonresistant loblolly pine, even when the soil moisture was at the wilting point. In the lowland prairie, where soil moisture was abundant, Marsh (8) found no correlation between leaf moisture and osmotic pressure in the species he studied. However, a relation occurred under the dry conditions in the upland prairie.

Several workers have indicated that a high sugar content is associated with drought resistance. Sorghums have a larger amount of reducing and nonreducing sugars than corn, according to Miller (13). As the intensity of drought increased, Vasiliev (16) found in wheat plants, first accumulation of sucrose; then hydrolysis of sucrose to monosaccharides; and finally, as complete desiccation occurred, disappearance of both sucrose and monosaccharides. Lvoff and Fichtenholz (6) observed that drought-resistant plants possess a greater power of hydrolyzing starch to monosaccharides than do susceptible plants.

Maximov (10) stated that a high osmotic pressure is generally characteristic of xerophytes and increases drought resistance by (1) protecting the protoplasm from coagulation and desiccation during wilting owing to the accumulation of salts and organic substances in the sap; (2) preventing wilting for a long time in the presence of a water deficit because of the tension on the mesophyll cells; and (3) enabling resistant plants to obtain moisture more readily from a very dry soil by creating a high suction power.

Martin (9) observed a higher osmotic pressure in the roots and stalks of sorghum than in those of corn, but the reverse in the case of the leaves. Newton and Martin (14) reported that generally a higher osmotic pressure was found in drought-resistant wheats and grasses than in those not resistant; however, they did not consider the osmotic pressure a major factor in determining drought resistance. Schopmeyer (15) arrived at similar conclusions. Meyer (11) concluded that the osmotic pressure could not be safely used in central Ohio to differ-

entiate between habitats. Korstian (5) found that moisture had a greater influence on the osmotic pressure of the cell sap than either light or temperature.

#### MATERIAL AND METHODS

The experiments reported here were confined to four varieties of spring wheat, namely Baart, Ceres, Marquis, and Hope, chosen because of their range in field behavior under drought conditions, presumably indicating differences in drought resistance. Baart is a white wheat, which generally produces a high grain yield in central Washington, in the high plateau lands of eastern Idaho, and in other far western locations where the yearly rainfall is below 15 inches. According to Clark (3), it is the most drought-resistant wheat grown in the United States. The other three are hard red spring varieties. Ceres has outyielded Marquis under the dry conditions of the northern Great Plains. Hope, although highly resistant to the wheat rusts, has been shown to be susceptible to drought injury under severe conditions.

Waldron (17) reported average yields of 16.3, 14.1, and 12.7 bushels per acre for Ceres, Marquis, and Hope, respectively, in 25 trials under generally dry, rust-free conditions in Montana and western North Dakota and South Dakota. Later (18) he reported Hope and its sibs to be very susceptible to hot, dry weather. Ceres was highly resistant to these conditions, and Marquis was intermediate.

These varieties were grown at the University Farm, Tucson, Ariz., under two environments established respectively in two comparable, adjacent large plots surrounded by levees, one plot irrigated and the other having no irrigation water applied after seeding and hence subjected to severe drought. These two environments are called the irrigated plot and the drought plot, respectively. The same plots were used each year. The irrigated plot was irrigated several times after seeding, as needed to keep the wheat growing in a normal condition so far as could be judged from the appearance of the wheat plants. The border method of irrigation was used throughout. The dates of these irrigations are shown later.

Since the field on which these plots were located is uniform and since the plots were adjacent to each other, it is a reasonable assumption that most of the differences in osmotic pressure, total solids, and leaf moisture are due directly or indirectly to differences in soil moisture. Some leaching of nitrates may have occurred in the irrigated plot during the growing season.

The soil on which these tests were conducted is classified as Gila loam, dark-colored phase, and has a moisture equivalent of 23.5 percent. The moisture content at the point of permanent wilting is 12.8 percent. The seedings were made on December 10, 1933, February 18, 1935, January 22, 1936, and January 26, 1938. No planting was made in 1937.

The first collections each year were made from plants in the irrigated plot. None was made in the drought plot until the plants showed a slight need for water. With the exception of 1935, the first collections from the drought plot were made soon after the plants in

the irrigated plot had received the first irrigation. In the early collections, when only one or two leaves of the wheat plants were fully extended, the samples for the laboratory determinations were gathered by cutting off the entire plant as close to the ground as possible. Later collections consisted of the top three leaves of the plants. When the second or third leaves from the top had started to dry in the drought plot, the samples from both plots consisted of only the top leaf or the top two leaves. The same sampling procedure was followed in both the irrigated and the drought plot. Final collections were made just before the top leaves in the drought plot had started to dry up. This was about 3 weeks or more before the plants reached maturity in the irrigated plot. No dead or partially dead leaves were collected at any time in either plot.

Except in 1934, the collections were started at 1:30 p. m. and generally were made every 4 days. The samples were taken to the laboratory and treated according to the method of Mallery (7) immediately after collection. A pressure of 10,000 pounds per square inch, obtained with a Carver hydraulic laboratory press, was used in expressing the cell sap from the heated leaf samples. A portion of each sample of sap was used for the determination of osmotic pressure by standard cryoscopic methods and the remainder for measuring the percentage of total solids with a refractometer.

For the leaf-moisture determinations in 1935 and in 1936, 2- to 4-gm. samples were dried to constant weight at 100° C. In 1938, 5- to 10-gm. samples were used and were dried at 80°. No determinations of leaf moisture were made in 1934.

Soil samples to a depth of 4 feet were taken for moisture determination in both the irrigated and the drought plot at three or more intervals during each of the 3 years. These samples were dried to constant weight at 100° C.

Observations made in the drought plot showed that in the last 3 years the crown roots were either lacking or were very weakly developed. Occasionally, when the leaves were pulled off the plants for cryoscopic determinations, entire plants would be pulled out of the ground. There seemed to be no varietal differences in this respect. Most of the plants had only a single culm. The plants in the drought plot were half to two-thirds the height of comparable irrigated plants at maturity. As a rule, the lower leaves of plants of the former were but slightly smaller than those of the latter. However, the top leaves in the drought plot were about half the size of those in the irrigated plot. All the varieties in the drought plot flowered each year, and some seed was produced each year except 1936. Yields, however, were very low because of severe drought injury. The plants of Baart flowered about 10 days earlier than those of Ceres, Marquis, and Hope. There was an abundance of waxy bloom on the leaves and leaf sheaths of Ceres. In the development of bloom the varieties ranked in the order Ceres, Baart, Marquis, and Hope. The wilting of the leaves was so gradual that it was difficult to observe any major changes. In the later stages, the top leaf in many Baart plants was entwined about the culm.

Bound-water determination made on samples of some of the plants gave such erratic results that the data are not reported.

## WEATHER CONDITIONS

The precipitation and mean temperature for each month from January to May for each of the years in which experiments were conducted are shown in table 1. The precipitation during the 4 years was lowest in 1934 and highest in 1935. There were no large differences in the mean monthly temperatures for the different years.

TABLE 1.—*Precipitation and mean temperature for each month during 4 growing seasons at Tucson, Ariz.*

Month	Precipitation				Mean monthly temperature			
	1934	1935	1936	1938	1934	1935	1936	1938
January	Inches	Inches	Inches	Inches	°F.	°F.	°F.	°F.
0.50	1.25	0.96	0.65	49.3	52.1	48.8	52.4	
February	.30	2.43	.92	.88	56.4	55.0	53.2	54.2
March	.39	1.46	.55	.43	63.9	55.0	59.0	57.6
April	.03	T	.07	.08	68.6	64.7	66.9	65.3
May	.05	.14	T	.11	78.4	67.6	75.3	71.3
Total or mean	1.27	5.28	2.50	2.15	63.3	58.9	60.6	60.2

<sup>1</sup> T=trace.

## SOIL MOISTURE

The percentages of soil moisture in the surface 4 feet of soil for 1935, 1936, and 1938 in the irrigated and drought plots are shown in table 2. Soil moisture in the irrigated plot was kept above the wilting percentage (12.8 percent) except in the surface foot, where it fell slightly below in 1936 and 1938.

With few exceptions the moisture content of the soil at the first samplings each year was about the same in the two plots, but at the last sampling dates for each season the moisture content of the soil in the drought plots usually was 0 to 7 percent less than in the irrigated plot. In the drought plots the soil moisture in the surface foot usually was below the wilting percentage except at the beginning of the season, but in the second, third, and fourth foot it usually was above.

## EXPERIMENTAL RESULTS

## MOISTURE CONTENT OF LEAVES

The moisture content of the leaves of all varieties, grown with and without irrigation, are shown in tables 3 and 4 and in figure 1. It will be noted that the deficit of soil moisture in the drought plot resulted in a considerable decrease in the moisture content of the leaves of all varieties as compared with those in the irrigated plot.

The average yearly difference in the percentage of leaf moisture between the irrigated and the drought plots for all sampling dates ranged from 1.6 for Ceres in 1935 to 6.8 for Marquis in 1938. There appeared to be no consistent differences between varieties in this respect. In 1935, the greatest difference (3.9 percent) was for Baart and the least (1.6 percent) for Ceres; in 1936, the greatest difference (5.0 percent) was for Marquis and the least (2.6 percent) for Baart; in 1938, the greatest difference (6.8 percent) again was for Marquis

and the least (4.0 percent) for Hope. In general, the differences increased as the season advanced and the soil-moisture deficit and need for water became greater.

TABLE 2.—*Soil moisture in irrigated<sup>1</sup> and drought plots at Tucson, Ariz.*

Date of sampling	Soil moisture under indicated treatment in—											
	Surface foot			Second foot			Third foot			Fourth foot		
	Irrigated	Drought	Difference <sup>2</sup>	Irrigated	Drought	Difference <sup>2</sup>	Irrigated	Drought	Difference <sup>2</sup>	Irrigated	Drought	Difference <sup>2</sup>
<i>1935</i>												
Mar. 12	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent
25	17	16	1	17	18	-1	21	20	1	24	22	2
25	14	15	-1	16	16	0	19	20	-1	22	21	1
Apr. 4	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent
14	17	14	3	20	17	3	22	19	3	24	20	4
14	17	13	4	19	16	3	22	18	4	22	20	2
24	15	12	3	20	15	5	23	18	5	20	20	0
May 1	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent
10	14	11	3	15	14	1	19	18	1	21	18	3
10	20	13	7	20	15	5	24	15	9	27	17	10
20	14	9	5	15	11	4	19	12	7	20	16	4
Average	16.0	12.9	3.1	17.8	15.3	2.5	21.1	17.5	3.6	22.5	19.3	3.3
<i>1936</i>												
Mar. 26	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent
15	15	15	0	17	17	0	18	18	0	20	20	0
Apr. 10	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent
17	12	5	18	13	5	19	13	6	23	18	5	4
26	11	11	0	14	11	3	13	10	3	15	11	4
May 10	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent
Average	14.3	12.7	1.7	16.3	13.7	2.7	16.7	13.7	3.0	19.3	16.3	3.0
<i>1938</i>												
Mar. 20	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent
16	16	11	5	17	17	0	20	17	3	21	18	3
Apr. 1	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent
13	11	2	16	15	1	16	16	0	23	18	5	5
11	10	4	16	13	3	18	15	3	22	15	7	7
18	14	10	4	13	12	1	18	14	4	21	17	4
25	12	10	2	17	11	6	17	13	4	24	18	6
May 5	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent
Average	13.8	10.4	3.4	15.8	13.6	2.2	17.8	15.0	2.8	22.2	17.2	5.0
3-year average	14.7	12.0	2.7	16.6	14.2	2.5	18.5	15.4	3.1	21.3	17.6	3.8

<sup>1</sup> Irrigated Mar. 30, Apr. 10, Apr. 25, and May 7, 1935; Mar. 26, Apr. 15, and May 9, 1936; and Mar. 23, Apr. 7, and Apr. 25, 1938. When plots were sampled on the day irrigated, the samples were taken first.

<sup>2</sup> Minus value indicates lower soil moisture in irrigated plot than in drought plot.

<sup>3</sup> Averages for comparable sampling dates only.

TABLE 3.—*Differences in moisture content of leaves in 4 varieties of wheat grown in irrigated<sup>1</sup> and drought plots at Tucson, Ariz.*

Date of sampling	Baart <sup>2</sup>	Ceres <sup>2</sup>	Marquis <sup>2</sup>	Hope	Date of sampling	Baart	Ceres	Marquis	Hope
1935	Percent	Percent	Percent	Percent	1936—Con.	Percent	Percent	Percent	Percent
Apr. 17	3	0	-2	1	Apr. 19	2	6	6	4
21	2	0	2	0	23	8	1	9	8
29	5	4	1	4	27	7	6	8	6
May 7	3	3	2	1	May 1	4	8	7	7
11	-1	-4	4	6					
15	5	2	2	1	1938				
19	10	6	7	6	Apr. 7	2	4	4	3
						11	5	6	3
						19	6	6	2
1936	-3	-1	1	2	23	5	6	8	4
Apr. 7	-1	-1	0	3	27	5	6	7	6
11	-1	3	4	3	May 5	8	8	8	6
15	1	3	4	3					6

<sup>1</sup> See footnote 1, table 2.

<sup>2</sup> Minus value indicates a higher leaf moisture in drought plot than in irrigated plot.

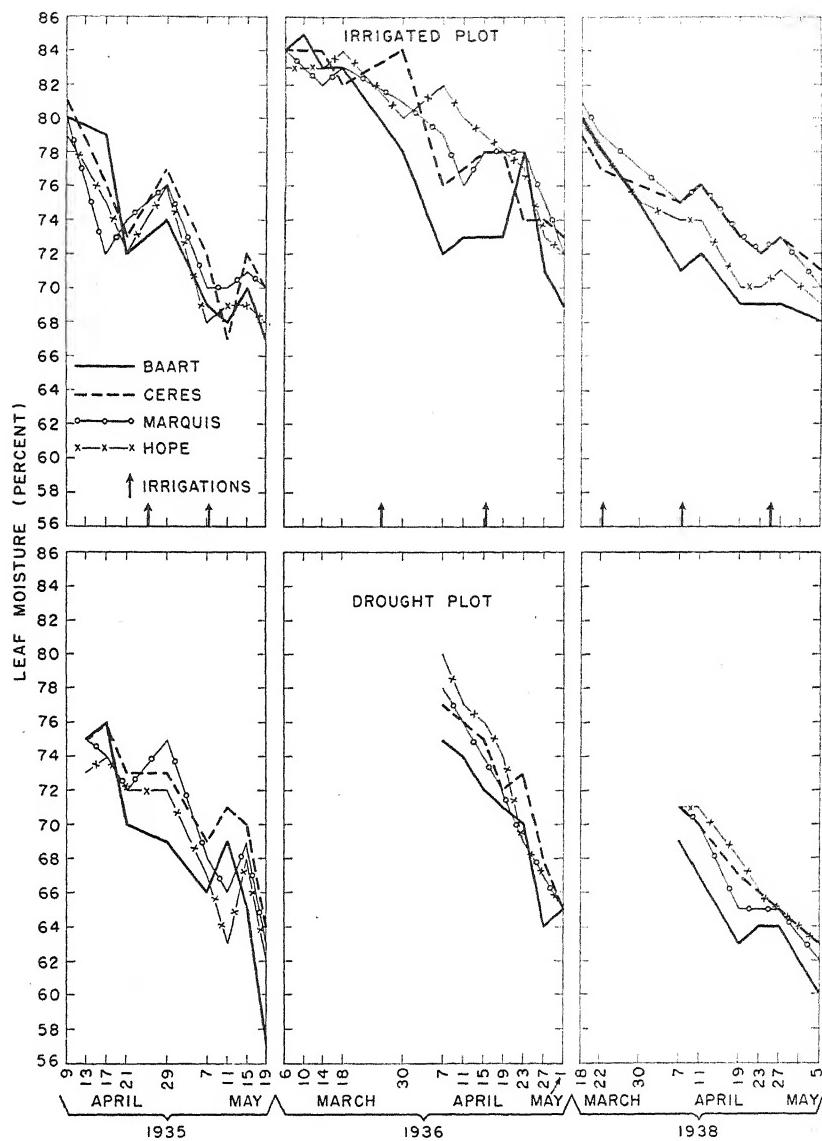


FIGURE 1.—Moisture content of leaves of four varieties of wheat grown in irrigated and drought plots at Tucson, Ariz.

TABLE 4.—*Average moisture content of leaves in 4 varieties of wheat grown in irrigated<sup>1</sup> and drought plots at Tucson, Ariz.*

Date of sampling	Moisture content of leaves under indicated treatment in—											
	Baart			Ceres			Marquis			Hope		
	Irrigated	Drought	Differ- ence	Irrigated	Drought	Differ- ence	Irrigated	Drought	Differ- ence	Irrigated	Drought	Differ- ence
Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent
1935.....	71.3	67.4	3.9	72.4	70.8	1.6	71.9	69.6	2.3	71.0	68.3	2.7
1936.....	72.7	70.1	2.6	75.7	72.3	3.4	76.6	71.6	5.0	77.3	72.6	4.7
1938.....	69.7	64.5	5.2	73.3	67.0	6.3	73.1	66.3	6.8	71.3	67.3	4.0
1935-38.....	71.2	67.3	3.9	73.8	70.0	3.8	73.9	69.2	4.7	73.2	69.4	3.8

<sup>1</sup> See footnote 1, table 2.

The principal interest in this study relates to differences between varieties on the drought plot. The moisture content of the leaves of Baart on the drought plot averaged lower than that of any other variety in each of the 3 years in which comparisons were made. This, however, appears not to have been altogether a result of the deficit in soil moisture, since there was a general tendency in the same direction for the irrigated plot. On the drought plot, Ceres had the highest 3-year average moisture content of the leaves. However, there was less than 1 percent difference between the 3-year averages of Ceres, Marquis, and Hope.

#### OSMOTIC PRESSURE OF LEAF JUICE

Corresponding data for osmotic pressures are given in tables 5 and 6 and in figure 2. As would be expected, the osmotic pressure of the

TABLE 5.—*Differences in osmotic pressure of leaf juices of 4 varieties of wheat grown in irrigated<sup>1</sup> and drought plots at Tucson, Ariz.*

Date of sampling	Baart	Ceres	Marquis <sup>2</sup>	Hope <sup>2</sup>	Date of sampling	Baart	Ceres	Marquis	Hope
1934	Atmos-	Atmos-	Atmos-	Atmos-	1936	Atmos-	Atmos-	Atmos-	Atmos-
Apr. 12	phères	phères	phères	phères	Apr. 7	phères	phères	phères	phères
19	5.20	5.24	2.58	3.95		2.58	3.24	2.88	3.44
29	7.49	5.35	2.62	3.49		11	1.44	1.20	1.14
	15.53	7.98	7.14	6.66		15	3.36	3.72	5.46
1935						19	6.36	7.26	7.80
						23	9.78	10.20	10.20
						27	12.10	6.47	8.39
Apr. 17	1.56	.36	1.32	-.42	May 1	15.67	10.30	8.27	10.43
21	1.92	2.58	.84	-2.04					
25	1.26	1.86	.72	-.84	1938				
29	1.56	2.46	.54	.48					
May 7	1.92	1.74	.48	-.16	Mar. 30	3.24	3.72	3.30	3.60
11	3.85	4.08	2.64	1.62	Apr. 3	4.52	3.23	3.06	1.66
15	5.70	4.32	2.58	.96		7	.15	.28	1.97
19	3.96	4.80	-.210	-.90		11	4.34	3.32	5.08
						19	6.24	4.96	5.32
						23	1.70	.84	.73
						27	6.63	3.04	3.60
						May 5	9.30	3.84	3.47
									3.92

<sup>1</sup> See footnote 1, table 2.<sup>2</sup> Minus value indicates a higher osmotic pressure in irrigated plot than in drought plot.

TABLE 6.—Average osmotic pressure of the leaf juices of 4 varieties of wheat grown in irrigated<sup>1</sup> and drought plots at Tucson, Ariz.

Date of sampling	Baart				Ceres				Marquis				Hope				
	Irrigated	Drought	Difference	Irrigated	Irrigated	Drought	Difference	Irrigated	Irrigated	Drought	Difference	Irrigated	Irrigated	Drought	Difference		
Osmotic pressure of leaf juice under indicated treatment in—																	
Atmos-	Atmos-	Atmos-	Atmos-	Atmos-	Atmos-	Atmos-	Atmos-	Atmos-	Atmos-	Atmos-	Atmos-	Atmos-	Atmos-	Atmos-	Atmos-	Atmos-	
pheres	pheres	pheres	pheres	pheres	pheres	pheres	pheres	pheres	pheres	pheres	pheres	pheres	pheres	pheres	pheres	pheres	
1934	11.82	27.33	15.51	6.19	21.70	6.19	16.97	21.08	16.97	4.11	21.05	4.70	16.76	21.86	5.10	16.86	
1935	16.68	19.35	2.72	19.08	2.78	16.35	17.23	6.88	16.92	15.00	22.20	6.55	19.28	22.48	3.20	19.30	
1936	17.35	24.68	7.33	16.32	22.38	6.06	15.65	22.78	3.10	19.28	6.86	19.28	22.78	3.50	19.30	22.78	
1938	20.14	24.66	4.52	19.43	22.33	2.90	19.68	22.78	3.10	19.28	6.86	19.28	22.78	3.50	19.30	22.78	
1934-38	17.99	23.98	5.99	16.89	21.37	4.48	17.16	20.82	3.66	16.89	20.54	3.65	16.89	20.54	3.65	16.89	20.54
1935-38	18.04	22.90	4.86	17.35	21.26	3.91	17.23	20.74	3.51	17.07	20.47	3.30	17.07	20.47	3.30	17.07	20.47

<sup>1</sup> See footnote 1, table 2.<sup>2</sup> Minus value indicates a higher osmotic pressure in irrigated plot than in drought plot.

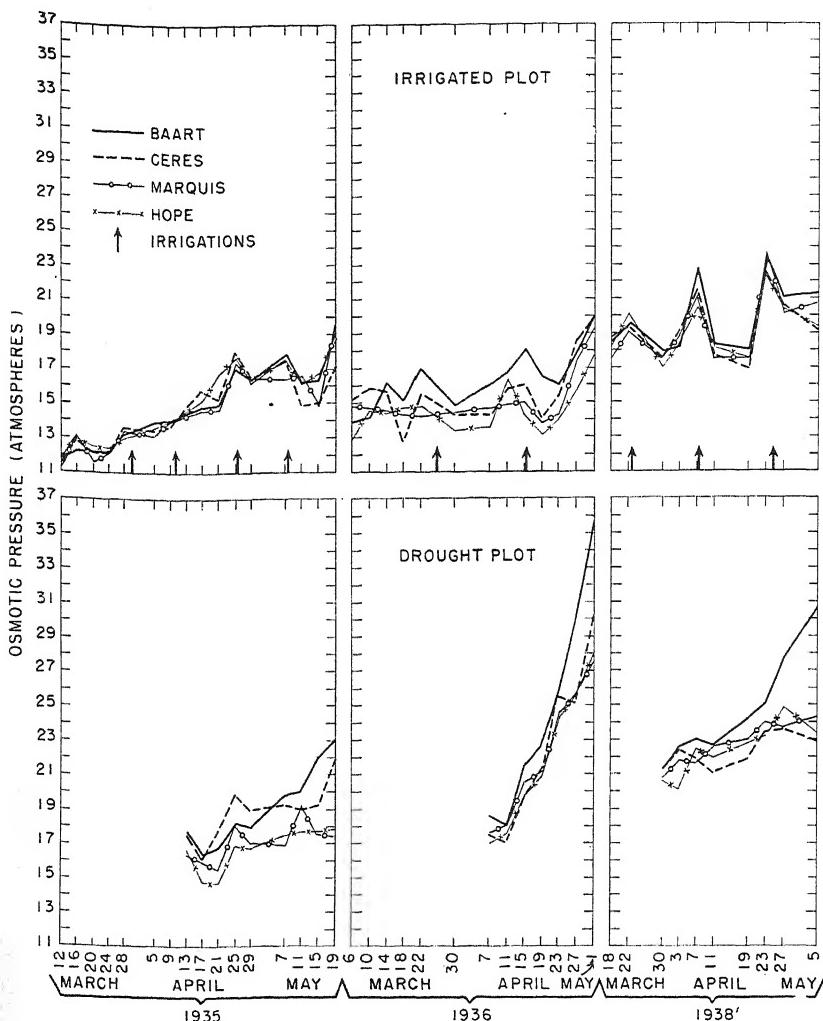


FIGURE 2.—Osmotic pressure of leaf juices of four varieties of wheat grown in irrigated and drought plots at Tucson, Ariz.

expressed leaf-blade juice was generally higher and in most cases considerably higher for the drought than for the irrigated plot. For the latter, Baart averaged higher than other varieties by about 1 atmosphere. The other three had substantially the same values. The differences, however, are not consistent from year to year, Baart averaging slightly less than Hope in 1935.

In the drought plot the osmotic pressures for Baart were rather consistently higher than those for other varieties at the later sampling dates and frequently so at the earlier dates also. As an average for all comparable sampling dates in all years, the four varieties fall into

the same order as for their assumed drought resistance, i. e., Baart, Ceres, Marquis, and Hope, the difference between Baart and Hope being 3.44 atmospheres (table 6).

#### TOTAL SOLIDS OF LEAF JUICE

The percentage of total solids in the expressed juice of the four varieties is shown in tables 7 and 8 and figure 3. As in the case of osmotic pressure, the total solids generally were highest for the plants in the drought plot. In the irrigated plot Baart averaged the highest and Hope the lowest for all comparable sampling dates in all years. They were also the highest and lowest, respectively, each year, though the differences in some cases were small.

TABLE 7.—*Differences in total solids of the leaf juices of 4 varieties of wheat grown in irrigated<sup>1</sup> and drought plots at Tucson, Ariz.*

Date of sampling	Baart	Ceres <sup>2</sup>	Marquis <sup>2</sup>	Hope <sup>2</sup>	Date of sampling	Baart	Ceres	Marquis	Hope
1934					1936				
Apr. 12	Percent	Percent	Percent	Percent	Apr. 7	Percent	Percent	Percent	Percent
19	2.73	2.48	1.34	1.28	11	.77	1.33	1.37	1.63
29	3.40	2.26	.68	.79	15	1.39	2.23	2.76	.98
					19	1.72	3.13	3.28	2.36
1935					23	2.87	2.25	3.99	2.87
Apr. 17	.72	-.15	-.33	.14	27	3.97	3.42	3.96	3.81
21	.50	.24	.37	-.32	May 1	6.08	4.03	3.89	3.39
25	1.63	.65	.81	1.21	1938				
29	1.08	.45	.54	1.04	Mar. 30	.77	1.47	1.55	1.34
May 7	2.36	.73	.33	.19	Apr. 3	1.75	2.03	2.22	1.69
11	2.44	1.60	1.59	.75		1.38	3.52	1.85	2.26
15	3.23	1.85	2.19	.86		11	2.29	2.35	3.31
19	3.02	2.13	1.54	.44		19	4.14	3.68	3.40
						23	2.97	1.89	3.05
						27	5.12	2.75	2.73
						May 5	7.63	4.54	3.62
									3.20

<sup>1</sup> See footnote 1, table 2.

<sup>2</sup> Minus value indicates a higher total solids from irrigated plot than from drought plot.

TABLE 8.—*Average total solids of the leaf juices of 4 varieties of wheat grown in irrigated<sup>1</sup> and drought plots at Tucson, Ariz.*

Date of sampling	Total solids of leaf juice under indicated treatment in—											
	Baart			Ceres			Marquis			Hope		
	Irrigated	Drought	Difference	Irrigated	Drought	Difference	Irrigated	Drought	Difference	Irrigated	Drought	Difference
1934.....	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent
1934.....	12.14	16.33	4.19	11.01	13.81	2.80	11.54	13.47	1.93	10.93	12.65	1.72
1935.....	10.98	12.85	1.87	10.94	11.88	.94	10.64	11.52	.88	10.40	10.94	.54
1936.....	9.42	11.89	2.47	9.13	11.73	2.60	8.80	11.69	2.89	8.40	11.07	2.67
1938.....	11.94	15.20	3.26	11.70	14.48	2.78	11.70	14.42	2.72	11.50	13.81	2.31
1934-38.....	11.12	14.07	2.95	10.70	12.98	2.28	10.67	12.78	2.11	10.31	12.12	1.81
1935-38.....	10.78	13.31	2.53	10.59	12.70	2.11	10.38	12.54	2.16	10.10	11.94	1.84

<sup>1</sup> See footnote 1, table 2.

TABLE 10.—*Interannual correlation coefficients for average osmotic pressure and average percentage of total solids of wheat leaf-blade juices from the drought plot*

Years compared	Osmotic pressure		Percentage of total solids	
	Number	<i>r</i>	Number	<i>r</i>
1934 and 1935	4	+0.710	4	+0.974**
1934 and 1936	4	+.902**	4	+.710
1934 and 1938	4	+.911*	4	+.988**
1935 and 1936	4	+.743	4	+.844
1935 and 1938	4	+.547	4	+.986**
1936 and 1938	4	+.982**	4	+.893*

\*\* Significant at the 1-percent level.

\* Significant at the 5-percent level.

Highly significant ( $P=0.01$ ) positive coefficients between osmotic pressure and percentage of total solids were found each year for both the irrigated and the drought plot. Of the five coefficients between soil moisture and leaf moisture, four were positive, three being highly significant. All other coefficients were negative or near zero, or both. Those between osmotic pressure and leaf moisture and between the percentages of total solids and leaf moisture were highly significant for both plots in all years.

In general, as would be expected, higher coefficients were found for the drought than for the irrigated plot, the differences in some cases being large. Thus, all coefficients for the drought plots but two were highly significant ( $P=0.01$ ) and of the two exceptions, one was significant ( $P=0.05$ ).

Of the six interannual coefficients (table 10) for osmotic pressure in the drought plot, two were highly significant and one was significant. Three of the coefficients for total solids were highly significant and one was significant. There appeared to be a tendency for the coefficients for total solids to be higher than those for osmotic pressure but not consistently so, that between 1934 and 1936 being distinctly less and that between 1936 and 1938 slightly less.

#### SUMMARY

The moisture content of the leaf blades and the osmotic pressure and percentage of total solids of the expressed juice of the leaf blades of four varieties of spring wheat, believed to differ in drought resistance and grown with and without irrigation (drought plot), were determined at several intervals in each of 3 years at Tucson, Ariz. The varieties studied were Baart, Ceres, Marquis, and Hope.

The data show that the deficit of soil moisture in the drought plot tended to decrease the moisture content of the leaves and to increase the osmotic pressure and percentage of total solids of the expressed juices.

The reduction of the moisture content of the leaves in the drought plot was about the same for all varieties, but the increase in osmotic pressure and in percentage of total solids was greatest in Baart and least in Hope. As an average for all years, the order of the varieties

with respect to osmotic pressure and percentage of total solids was the same as their supposed drought resistance: namely, Baart, Ceres, Marquis, and Hope. The average moisture content of the leaves was lowest for Baart and highest for Ceres, the differences between all varieties other than Baart being small and inconsistent from year to year.

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## THE RESPONSE OF HYBRID POPLAR AND OTHER FOREST TREE SPECIES TO FERTILIZER AND LIME TREATMENT IN CONCRETE SOIL FRAMES<sup>1</sup>

By HERBERT A. LUNT

*Associate in forest soils, Connecticut (New Haven) Agricultural Experiment Station*

### INTRODUCTION

A set of 48 concrete-walled soil frames or bins which had been used 7 years for a wide variety of crops was turned over to the writer in the spring of 1934 for use in forest tree studies. The fertility level varied greatly as a result of the previous treatments, and crop responses had been most striking. The frames provided opportunity for experimenting with trees in what were essentially pot culture tests, and the object of the study was to observe the behavior of the trees under reasonably controlled conditions where presumably the only variable was the nutrient supply. Special emphasis is placed on the results with hybrid poplar inasmuch as the stock came from a single clone of known genetic history.

### LITERATURE REVIEW

The literature on fertilization of nursery stock and plantations is quite voluminous and in many cases contradictory. Much of the work done abroad is inapplicable to conditions in this country. The apparent discrepancies in the responses reported may be ascribed to differences in species, source of seed or planting stock, kinds of fertilizers, method and rate of application, weather conditions, fertility level, and physical condition of the soil. There is ample evidence that red pine is relatively unresponsive to fertilizers (*9, 20*).<sup>2</sup> Results on spruce obtained by the writer (unpublished) and others frequently lack agreement, but the consensus of opinion is that the requirements of Norway, white, and probably red spruce are higher than those of red or Scotch pine.

In the report of a special committee of the New York Section of the Society of American Foresters (*7*), it was stated that most hardwood and shrub species require better soils and are less able to withstand weed and grass competition than the average conifer. Red oak can grow almost anywhere once it is established, but sugar maple requires a fairly good site.

<sup>1</sup> Received for publication January 3, 1946.

<sup>2</sup> Italic numbers in parentheses refer to Literature Cited, p. 131.

There is reasonably good evidence (4, 8, 11) that growth of black locust and green ash is increased by fertilizers high in phosphorus and low in nitrogen and potassium. Cummings (3) found that growth differences with white ash were small but that there was a generally beneficial effect from the highest application of nitrogen. Yellow poplar showed no significant response to any treatment.

Very little work has been done on the nutritional requirements of hybrid poplars. Schreiner (16) found that they are particularly sensitive to inimical environmental conditions, and that the beneficial effect of sod, turned under, far exceeded that of fertilizers, even where comparatively large quantities of nitrogen were used.

## EXPERIMENTAL PROCEDURE

### SOIL FRAMES, TREATMENTS, AND PREVIOUS HISTORY

The trees were grown in 3 sets of 16 frames, each frame being approximately 2 feet square (0.0001 acre) and 2 feet deep, without artificial bottoms, resting directly on the natural subsoil. They were constructed and filled in 1927, with Cheshire loam.<sup>3</sup>

Treatments were 0, P, K, PK, N, NP, NK, and NPK, and the same repeated with lime.<sup>4</sup> The initial liming in 1927 increased the soil pH from 5.0 to 7.5 on the limed series. By 1939 it had dropped to 5.6, and the soils of the acid series averaged 4.75. Sufficient limestone was then added to raise the pH of the acid series to 5.2 and that of the limed series to 6.6. Beginning with 1939, calcium acetate was included with all nitrogen treatments in order to lessen the danger of calcium deficiency. Details of the treatments and the cropping history are given in table 1, and the arrangement of the frames is shown in figure 1.

Choice of fertilizer materials was prompted by the desire to avoid accumulating nonessential ions, such as sodium, chlorine, etc., in the soil. The soil test data given in table 2 show the fertility status of the soil. It will be noted that all soils receiving nitrogen were more acid because of the nitric acid formed and the absence of bases to neutralize it. Nitrate nitrogen tests are not listed because they almost invariably showed low values as a result of the rapid utilization of nitrates by the growing crops. Phosphorus and potassium differences were extreme because of the long period of treatment. Calcium was increased greatly by lime. In all cases where the treatment included N but left out K, the Ca content was conspicuously low because Ca unites with nitric acid in the soil to form calcium nitrate which is either absorbed by the roots or leached downward. The same condition applies to magnesium but to a lesser degree. With the inclusion of potassium in the treatment, this role of neutralizer is shared by the potassium, hence the drain on calcium and

<sup>3</sup> Reddish-brown upland soil derived from Triassic sandstone and shale glacial till. The original profile consisted of 6 to 8 inches of dark-brown loam (A), overlying a slightly reddish yellow-brown sandy loam subsoil (B), which at 24 inches, graded into a coarse, stony, reddish glacial till (C). Only the A and B horizons were used to fill the frames, the soil from each horizon being thoroughly mixed separately and placed in the frames in the same relative positions as in the field.

<sup>4</sup> 0 = check; P = phosphorus; K = potassium; N = nitrogen; L = lime.

TABLE 1.—Fertilizer and lime treatments given and cropping history of soil frames used in experiments

Growing season	Materials and rate of application			Crops		
	Element or lime	Carrier	Pounds per acre	West series (frames 1-16)	Middle series (frames 17-32)	East series (frames 33-48)
1927						
1928	N	Urea	100 N		Celery	
1929	P	H <sub>2</sub> PO <sub>4</sub>	200 P <sub>2</sub> O <sub>5</sub>		Sweet corn	
1930	K	K acetate	100 K <sub>2</sub> O		Spinach—tomatoes—rye	
1931	L <sup>1</sup>	CaCO <sub>3</sub>	8,000 CaCO <sub>3</sub>		Lettuce—peppers—rye	
1932			(Identical all 7 yrs. except for lime)		Dahlias	
1933					Havana seed tobacco	
					Barley—buckwheat—rye	
1934						
1935						
1936						
1937						
1938						
1939	N	{ Urea Ca acetate	{ 100 N 30 Ca			
	P	CaH <sub>4</sub> (PO <sub>4</sub> ) <sub>2</sub>	200 P <sub>2</sub> O <sub>5</sub>			
	K	KCl	100 K <sub>2</sub> O			
	Mg <sup>2</sup>	MgSO <sub>4</sub>	60 MgO			
	L <sup>3</sup>	CaCO <sub>3</sub>	Varied			
			(Half in spring, half in fall)			
1940	N	{ Uramon Ca acetate	{ 25 N 30 Ca			
		(Spring only)				
1941	N	{ Urea Ca acetate	{ 25 N 15 Ca			
	P	CaH <sub>4</sub> (PO <sub>4</sub> ) <sub>2</sub>	50 P <sub>2</sub> O <sub>5</sub>			
	K	KCl	25 K <sub>2</sub> O			
	Mg <sup>2</sup>	MgSO <sub>4</sub>	25 MgO			
			(Spring only)			
1942						
1943						

<sup>1</sup> Lime applied only twice—1927 and 1939.<sup>2</sup> Magnesium applied to all frames.<sup>3</sup> Enough CaCO<sub>3</sub> added to bring soils to uniform pH—5.2 for acid series, 6.6 for limed series. Applied in spring only.

TABLE 2.—Average values of soil acidity and quick tests in soils after treatments with different fertilizer elements and lime

[Universal quick-test method (12)]

Treatment <sup>1</sup>	pH	Pounds per acre of—					Treatment <sup>1</sup>	pH	Pounds per acre of—				
		P	K	Ca	Mg	Al			P	K	Ca	Mg	Al
0	4.98	15	90	600	15	450	L	6.06	30	140	3,400	60	80
P	4.87	120	100	530	25	310	LP	5.79	15C	100	3,370	80	50
PK	5.16	120	535	760	50	230	LPK	6.09	20C	650	3,100	90	25
K	5.29	15	555	780	30	275	LK	6.28	30	610	3,460	60	30
NK	4.60	15	195	270	15	500	LNK	5.50	20	575	2,170	40	200
N	4.39	20	85	260	10	510	LN	5.19	20	120	1,470	25	270
NP	4.35	160	85	270	10	400	LNP	5.25	150	140	1,890	40	185
NPK	4.59	160	200	430	15	375	LNPK	5.43	150	575	2,500	60	125
Average	24.78	78	231	480	21	381	Average	25.70	94	364	2,670	57	121

<sup>1</sup> L=lime.<sup>2</sup> According to Dr. C. I. Bliss, Connecticut station biometrist, the arithmetic mean of the pH values comes nearer to describing the middle of the group than does the pH calculated from the mean of the hydrogen-ion concentrations.

magnesium is lessened. Aluminum tended to vary inversely with calcium and the pH values.

It should be emphasized that the soil differences existing during the time when the trees occupied the ground and the effects of these differences on tree growth were due in part to the residual effect of previous treatments and in part to the current treatment.

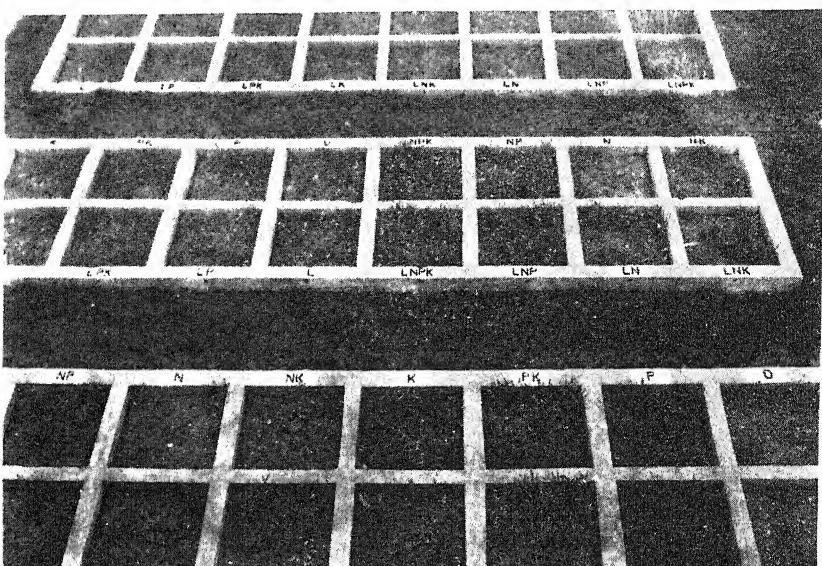


FIGURE 1.—View of soil frames, showing rye cover crop just prior to spading under in preparation for planting red pine, red oak, and sugar maple. Upper set—west series; middle set—middle series; lower set—east series. Photographed April 21, 1934.

Unfortunately, treatments were not randomized, and close proximity to greenhouses and a 2½-story building caused partial shade and an unevenness of snow cover resulting from interference with air currents. The earlier work reported in part in previous publications (9, 10) was more or less exploratory in nature and hence was not always replicated. It will be presented very briefly, most emphasis being placed on the hybrid poplars.

#### KIND AND SOURCE OF STOCK

Red pine (*Pinus resinosa* Ait.) hand-selected 2-0 stock, obtained from a local nursery, was planted 30 trees to the frame, and 2 years later thinned to 9 to the frame. Southern red oak (*Quercus falcata* Michx.) and sugar maple (*Acer saccharum* Marshall) 6- to 12-inch seedling whips were obtained from Tennessee and planted 5 to the frame. The oaks were cut back to 3 inches, and after 2 years, thinned to 1 tree per frame. At the end of 4 years the maples were thinned to 3 per frame. One-year-old white ash (*Fraxinus americana* L.) seedlings from a Connecticut State nursery were planted on 2 series, 5 trees to the frame. Norway spruce (*Picea excelsa* Link) 1-0 selected seedlings, from a State nursery, were planted 30 to the frame. White

spruce (*Picea glauca* (Moench) Voss) seed, procured from a dealer, was sown at the rate of 6.25 gm. to the frame.

The hybrid poplar cuttings used in this work were from nursery stock grown at Williamstown, Mass.<sup>5</sup> All the cuttings came from a single hybrid clone designated as OP-8. The parentage of this hybrid is *Populus nigra* L. (female)  $\times$  *P. laurifolia* Ledeb. (male). The use of dormant, graded cuttings of a single clone eliminates most of the initial variation (due to size and inherent variability) to be expected from seedling stock (15).

The cuttings were 12 inches long and graded as to diameter. Total weight of the six cuttings planted in each frame ranged from 65 to 77 gm. with an average of 71.3 gm. Two buds were left above ground in all cases. The first application of fertilizer was made April 2; the cuttings were planted April 5, with five replacements on May 18 from extra cuttings which had been started with the others. The second application of fertilizer was made June 14. Water was applied twice during the unusually dry weather of late summer.

On August 17, one tree of average size was removed from each frame and plant tissue tests were run on the petioles. Green and dry weights were obtained on the bare stems. On October 11, petioles were removed from the upper third or half of each tree (except 18 trees saved for subsequent rooting tests) and submitted to plant tissue tests. On October 29-30, all remaining leaves were stripped off, the whips cut off, and green and dry weights obtained. The 18 trees previously mentioned were weighed green and then placed in cold storage. In January 1944, cuttings from these were set in moist sand to determine whether previous treatment had affected rooting and sprouting ability.

#### METHODS OF ANALYSIS

Chemical analysis of oak, maple, and ash leaves were made by the usual laboratory methods<sup>6</sup> on dried and ground leaves including petioles. In the case of the poplars, plant tissue tests were run on the fresh petioles by a modification of the method proposed by Wolf (21), which in turn is an adaptation of Morgan's Universal Soil Testing System (12), to permit readings by means of the photelometer.

Soil tests were made by Morgan's method (12), except for the October 1943 sampling, when a modification of the Wolf (21) method was used.

### RESULTS WITH PINE, OAK, MAPLE, ASH, AND SPRUCE

#### RED PINE

Detailed data pertaining to the results with red pine have already been presented (9), and will be summarized only briefly here. At the end of the third season differences due to treatment were relatively small (table 3). Growth was definitely poorer with lime in seven cases out of eight. Potassium appears to have been beneficial, but

<sup>5</sup> The cuttings were obtained through the courtesy of Dr. E. J. Schreiner, of the Northeastern Forest Experiment Station.

<sup>6</sup> Total nitrogen by the Gunning method modified to include nitrates. Ash constituents by the perchloric acid digestion of Gieseking, Snider, and Getz (6).

weights taken on trees removed the preceding year during a thinning operation (data not given) showed no such indication. In six cases out of eight, the root-top ratio was lower on the limed soils (averages, limed 0.190; unlimed 0.209), and varied among the individual treatments from 0.166 to 0.241. Data obtained more recently in nursery studies of other conifers have been very inconsistent with respect to root-top ratios. No chemical analyses were made of the red pines.

TABLE 3.—*Response of red pine and southern red oak to soil treatment with fertilizer elements and lime*

RED PINE

Soil with—	Average dry weight per tree (tops and roots) <sup>1</sup>								Average
	0	P	PK	K	NK	N	NP	NPK	
No lime.....	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>
40.9	36.3	37.8	50.4	49.1	45.8	45.7	44.2	43.8	
Lime.....	35.5	37.0	33.0	42.9	46.3	40.4	39.6	38.8	
Average.....	38.2	36.7	35.4	46.7	47.7	43.1	42.7	41.5	
Relative.....	100	96	93	122	125	113	112	109	

SOUTHERN RED OAK

Soil with—	Average green weight per tree (tops only) <sup>2</sup>								Average
	157	216	172	220	213	348	133	246	
No lime.....	157	216	172	220	213	348	133	246	213
Lime.....	136	129	203	139	145	140	276	251	177
Average.....	147	173	188	180	179	244	205	249	
Relative.....	100	118	128	122	122	166	140	170	

<sup>1</sup> Based on 8-10 trees per frame, April 1937.

<sup>2</sup> Based on 4 or 3 trees in 1936, 1 tree in 1937.

SOUTHERN RED OAK

Typical of hardwoods, the individual red oak trees varied greatly in rate of growth. Obviously the small number of trees in each frame and the absence of replication seriously lower the validity of the data (table 3). However, certain trends may be noted: (1) growth averaged less on the limed soils, and (2), there was a general, if inconsistent, response to treatment. It is believed that the low value for NP and the very high value for N, both without lime, were decidedly abnormal and cannot be attributed to the treatments.

Chemical analysis of the leaves collected in the fall of 1935 showed relatively small differences. The data are summarized in table 4. The lower values for silica on the limed soils indicate that the calcium either repressed the absorption of silica or took its place to a certain extent. The same can be said in explanation of the slightly lower potassium content where lime was used. Phosphorus treatment resulted in a somewhat higher phosphorus content. In the absence

of phosphorus, silica absorption was increased on the unlimed soils. Potassium applied to the soil was not reflected in any increase in potassium in the leaves.

TABLE 4.—*Composition of southern red oak leaves collected in the fall of 1935 from trees in limed and unlimed soil*

[Average values for 8 treatments]

Constituent	Without lime		With lime	
	Average	Range	Average	Range
	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>
Ash.....	4.89	4.32-5.19	4.85	4.39-5.25
N.....	1.98	1.85-2.17	2.04	1.86-2.17
P.....	.14	.13-.16	.16	.14-.17
SiO <sub>2</sub> .....	.60	.49-.72	.44	.33-.55
K.....	1.11	.96-1.24	1.00	.92-1.12
Ca.....	1.03	.84-1.16	1.20	.98-1.47

#### SUGAR MAPLE

Like the oaks, the sugar maples were decidedly lacking in uniformity of growth. The data in table 5 show a positive response to lime in

TABLE 5.—*Response of sugar maple to soil treatments with fertilizer elements and lime*

[Average green weight per tree, exclusive of roots; based on 3 trees, 1939]

Soil with—	0	P	PK	K	NK	N	NP	NPK	Average
	Grams								
No lime.....	160	467	280	185	384	190	214	180	257
Lime.....	254	407	203	372	462	402	330	316	343
Average.....	207	437	242	279	423	296	272	248	—
Relative.....	100	211	117	134	204	143	131	120	—

most cases. In general, growth was increased by fertilizer treatment, but no attempt is made to explain why the largest trees were those receiving the P and NK treatments, and next to the smallest those receiving the NPK treatment.

Leaf samples were collected and analyzed separately in 1935, 1936, and 1938. Detailed data are given in table 6. The difference between years was highly significant for ash, N, K, and Ca; differences between treatments were all significant except those for nitrogen. Variations due to applied nitrogen were significant only for nitrogen, those due to lime were highly significant for ash and calcium, but not for phosphorus. The ash content was significantly affected also by phosphorus (directly) and potassium (inversely); potassium by the potassium treatment (directly), and calcium by the potassium treatment (inversely) as well as by lime (directly).

TABLE 6.—*Composition of sugar maple leaves from trees in soils treated with different fertilizer elements and lime*

## MEANS OF 1935, 1936, AND 1938 ANALYSES

Treatment <sup>1</sup>	Ash	N	P	SiO <sub>2</sub>	K	Ca
	Percent	Percent	Percent	Percent	Percent	Percent
0	6.65	1.64	0.117	1.87	0.71	1.38
P	7.57	1.69	.153	2.19	.77	1.62
PK	6.89	1.68	.186	2.23	.91	1.25
K	6.63	1.66	.124	1.94	.94	1.31
NK	6.84	1.74	.150	1.94	1.09	1.24
N	7.31	1.79	.144	2.27	.88	1.34
NP	7.70	1.67	.136	2.28	.82	1.56
NPK	6.60	1.73	.140	2.26	1.00	.97
Average	7.03	1.70	.144	2.12	.89	1.33
L	7.76	1.55	.125	1.88	.68	1.85
LP	8.43	1.66	.224	2.45	.79	1.76
LPK	7.98	1.64	.256	2.01	.97	1.64
LK	7.55	1.74	.152	2.15	.95	1.46
LNK	7.08	1.66	.127	1.76	.91	1.61
LN	7.47	1.81	.132	2.01	.74	1.78
LNP	7.94	1.85	.143	2.27	.76	1.95
LNPK	7.03	1.74	.193	1.71	.98	1.57
Average	7.66	1.71	.169	2.03	.85	1.70

## MEANS OF ALL TREATMENTS

Year:						
1935	6.47	1.94	.161	2.14	.93	1.48
1936	7.63	1.59	.156	1.96	.81	1.45
1938	7.93	1.59	.152	2.17	.87	1.62

SIGNIFICANCE OF VARIATIONS DUE TO YEARS AND TREATMENTS<sup>2</sup>

Years	**	**	—	—	**	**
Treatments	**	—	**	*	**	**
N	—	*	—	—	—	—
L	**	—	—	—	—	**
P	*	—	**	#	—	—
K	**	—	—	—	**	**
NP	—	—	*	—	—	—

<sup>1</sup> L=lime.<sup>2</sup> \*\*Highly significant; \*significant; #almost significant; —not significant.

## WHITE ASH

As white ash was grown on two series, the data were subjected to statistical analysis. From table 7, it is seen that there were significant differences between treatments, and that variations due to nitrogen and to lime were highly significant. On the basis of average values, NP produced the largest trees, but statistically the effects of P were not significant.

The means of the 1938 and 1939 leaf analyses are presented in table 8. Oddly enough, the leaves from the limed frames were practically no higher in calcium than those without lime, but their calcium content was significantly affected by N directly, and by K inversely. Nitrogen applications resulted in a higher concentration of nitrogen in the leaves on the unlimed plots only. The phosphorus content was increased by the phosphorus treatment, particularly on the plots without nitrogen. Plants receiving phosphorus had a tendency to take up more SiO<sub>2</sub> than those without phosphorus. The higher potassium concentration from the K-treated frames was almost significant.

Magnesium was affected directly by the lime and inversely by the potassium treatments.

During the 1938 growing season it was noticed that a considerable number of leaves were definitely crinkled and others had a dark mottled appearance, neither of which could be correlated with soil treatment. Analysis of such leaves shows (bottom of table 8) that

TABLE 7.—*Response of white ash trees to soil treatment with fertilizer elements and lime*

[Average green weight per tree, exclusive of roots, at the end of the third growing season, 1939; based on 5 trees per frame]

Soil with—	Series	0	P	PK	K	NK	N	NP	NPK	Total
No lime	West	Grams 152	Grams 73	Grams 127	Grams 90	Grams 136	Grams 121	Grams 128	Grams 184	Grams 1,123
	Middle									982
	Total		225	256	215	219	314	251	341	2,105
Lime	West	Grams 94	Grams 84	Grams 134	Grams 118	Grams 127	Grams 80	Grams 223	Grams 163	Grams 225
	Middle									1,366
	Total		178	252	218	208	436	393	500	2,658

#### ANALYSIS OF VARIANCE

Source of variation	Degrees of freedom	F	Source of variation	Degrees of freedom	F
Between series	1	1.46	K	1	<1
Between treatments	15	15.23	PK	1	2.64
N	1	147.11	NK	1	<1
L	1	19.66	NP	1	<1
P	1	3.13	LN	1	14.23

<sup>1</sup> Highly significant.

TABLE 8.—*Composition of white ash leaves from trees in soils treated with different fertilizer elements and lime*

#### MEANS OF 1938 AND 1939 ANALYSES

Treatment	Ash	N	P	SiO <sub>2</sub>	K	Ca	Mg
	Percent	Percent	Percent	Percent	Percent	Percent	Percent
0	5.87	1.14	0.127	0.605	1.49	1.19	0.126
P	6.39	1.05	.213	.715	1.60	1.05	.092
PK	6.79	1.16	.236	.925	1.67	.93	.099
K	6.02	1.09	.115	.575	1.67	1.05	.074
NK	6.49	1.58	.139	.605	1.83	1.11	.061
N	6.86	1.39	.115	.585	1.81	1.27	.069
NP	7.15	1.43	.171	.800	1.80	1.28	.095
NPK	6.79	1.30	.174	.695	1.94	.93	.071
Average	6.55	1.27	.161	.688	1.73	1.09	.086
Lime	6.64	1.19	.153	.570	1.63	1.15	.151
LP	7.11	1.21	.252	.590	1.61	1.13	.143
LPK	6.69	1.43	.301	.530	1.65	.93	.121
LK	6.87	1.21	.173	.500	1.75	1.01	.089
LNK	7.31	1.17	.124	.495	1.87	1.19	.091
LN	6.35	1.18	.127	.555	1.37	1.21	.142
LNP	6.39	1.21	.175	.565	1.27	1.31	.150
LNPK	6.61	1.37	.172	.575	1.71	1.15	.105
Average	6.75	1.25	.183	.547	1.61	1.13	.124

TABLE 8.—*Composition of white ash leaves from trees in soils treated with different fertilizer elements and lime—Continued*

## MEANS OF ALL TREATMENTS

Treatment <sup>1</sup>	Ash	N	P	SiO <sub>2</sub>	K	Ca	Mg
	Percent	Percent	Percent	Percent	Percent	Percent	Percent
Year:							
1938.....	7.10	1.44	0.185	0.551	1.73	1.04	0.088
1939.....	6.19	1.07	.161	.683	1.61	1.19	.122

SIGNIFICANCE OF VARIATIONS DUE TO YEARS AND TREATMENTS<sup>2</sup>

Years.....	**	**	*	**	*	*	**
Treatments.....	θ	—	**	—	**	—	**
N.....	—	—	θ	—	—	—	—
L.....	—	—	—	**	—	—	**
P.....	—	—	—	*	—	—	—
K.....	—	—	—	—	θ	**	**

## SPECIAL SAMPLES 1938 ONLY

Normal leaves.....	6.35	1.99	0.193	0.377	1.51	1.08	0.115
Crinkled leaves.....	8.59	1.52	.186	.575	1.62	1.42	.099
Dark mottled leaves.....	7.55	1.31	.166	.563	1.86	.97	.072

<sup>1</sup> L=lime.<sup>2</sup> \*\*Highly significant; \*significant; θ almost significant; — not significant.

the abnormal leaves were lower in N, P, and Mg, and higher in SiO<sub>2</sub> and K than the normal. Whether these differences are significant is not known.

## NORWAY SPRUCE

Data obtained at the end of one season (table 9) showed that growth of Norway spruce was less on the limed soils in 5 cases out of 8. Growth was poorest in the K-treated frames. The highest proportion of roots to tops was found in the NP frame, and the lowest in the K frame. These data are based on an average of 23 plants per frame, with 1 frame, NPK, having only 8 plants. No significance is attached to these differences; the data are given primarily for record.

TABLE 9.—*Response of Norway spruce to soil treatment with fertilizer elements and lime, one season only, 1939*

[Number of plants per frame ranged from 8 to 30, with an average of 23]

Soil with--	Average dry weight of whole plant (tops and roots)								Average
	0	P	PK	K	NK	N	NP	NPK	
No lime.....	Grams 0.67 .52	Grams 0.84 .51	Grams 0.80 .46	Grams 0.40 .45	Grams 0.70 .61	Grams 0.36 .65	Grams 0.68 .63	Grams 0.65 .70	Grams 0.637 .566
Lime.....									
Average.....	.595	.675	.63	.425	.655	.505	.655	.675	.601
Root-top ratio									
No lime.....	0.500 .598	0.526 .649	0.597 .554	0.373 .673	0.703 .669	0.787 .765	0.823 .716	0.573 .631	0.610 .657
Lime.....									
Average.....	.549	.587	.575	.523	.686	.771	.769	.602	.633

## WHITE SPRUCE

Full replication permitted statistical analysis of the data on white spruce, although the experimental design was systematic rather than randomized. Survival was extremely variable owing to a combination of factors most of which were independent of treatment. The data in table 10 show that lime had no effect on survival.

The size of plants, based on average green weight of the whole tree, including roots, at the end of the second year was smaller on the limed series. Among individual treatments, LK and LNK produced the smallest trees, and PK, NP, NPK, and LNPK the largest. Statistical analysis (table 11) reveals that differences due to L, P, and PK were

TABLE 10.—Number of white spruce plants per frame and average green weight per tree

Soil with—	Series	Number of plants per frame								Total
		0	P	PK	K	NK	N	NP	NPK	
No lime	West	54	1	38	51	208	214	188	131	885
	Middle	127	403	267	165	228	170	296	340	1,996
	East	246	364	253	225	235	130	236	261	1,956
Total		427	768	558	441	671	520	720	732	4,837
Lime	West	63	111	35	159	186	147	304	183	1,188
	Middle	388	141	225	0	61	215	281	314	1,625
	East	239	242	281	304	335	254	216	180	2,051
Total		690	494	541	463	582	616	801	677	4,864
Grand total		1,117	1,262	1,099	904	1,253	1,136	1,521	1,409	9,701
Average green weight per tree (tops and roots)										
No lime	West	.73	.80	1.59	.83	.91	1.15	1.80	2.05	9.86
	Middle	1.18	1.27	1.51	.93	1.16	1.57	1.55	1.71	10.88
	East	.74	.95	1.28	.71	1.19	1.00	.71	.79	7.32
Total		2.65	3.02	4.33	2.47	3.26	3.72	4.06	4.55	28.06
Lime	West	.49	.72	1.14	.37	.27	.32	1.22	1.42	5.95
	Middle	.70	1.48	1.20	0	.20	.95	1.22	1.68	7.43
	East	.46	.69	.73	.61	.42	.73	.64	1.01	5.29
Total		1.65	2.89	3.07	.98	.89	2.00	3.08	4.11	18.67
Grand total		4.30	5.91	7.40	3.45	4.15	5.72	7.14	8.66	46.73
Relative		100	137	172	80	97	133	166	201	—

TABLE 11.—Analysis of variance for average green weight per tree (white spruce), corrected by covariance for differences in stand

[Based on data in table 10]

Variation due to—	Degrees of freedom	Mean square (reduced [ $\bar{Y}^2$ ])	Observed F
Blocks	2	0.607690	17.98
Lime	1	1.848082	14.27
Nitrogen	1	.258040	3.38
Phosphorus	1	2.202344	12.93
Potassium	1	.019411	<1
Phosphorus $\times$ potassium	1	.657345	18.64
Lime $\times$ phosphorus	1	.385563	5.06
Interactions	9	.038517	<1
Regression of yield on stand	1	.282745	3.71
Blocks $\times$ treatments (error)	29	.076136	—
Total	47	—	—

<sup>1</sup> Significant.

significant, and that LP would undoubtedly have been significant had the treatments been randomized. The analysis of variance was corrected by co-variance for differences in stand.

There was considerable variation in the root-top ratios on the different frames but only those differences due to lime were significant (average without lime 0.586; with lime 0.633).

No analyses were made of the white spruce plants.

### RESULTS WITH HYBRID POPLAR

#### GROWTH

The oven-dry ( $73^{\circ}\text{C}.$ ) weights of the trees, minus leaves and original cuttings, are given in table 12. Growth differences were quite pronounced, with nitrogen causing greatest response, lime next, and then phosphorus. Combinations of two or more elements were generally more effective than any one element alone. The greatest average increase was 275 percent. Potassium was inconsistent in its effect, and in many cases growth was less with it than without. Similar behavior was observed in spruce. The significance of these differences is shown in table 13.

TABLE 12.—*Average dry weight per tree of hybrid poplars treated with different fertilizer elements and lime<sup>1</sup>*

Block	O	P	PK	K	NK	N	NP	NPK	Total
	Grams								
West	13.9	14.2	14.7	13.6	31.7	57.9	49.5	49.7	245.2
Middle	14.3	22.8	12.8	12.7	25.6	21.7	235.5	38.1	183.5
East	15.8	22.1	13.3	15.5	25.7	31.0	30.7	36.3	190.4
Average	14.7	19.7	13.6	13.9	27.7	36.9	38.6	41.4	619.1
Relative	100	134	92	94	188	250	263	282	-----
	L	LP	LPK	LK	LNK	LN	LNP	LNPK	Total
West	17.3	11.8	17.8	16.6	41.2	43.0	63.8	53.4	262.9
Middle	19.4	23.2	21.4	20.1	59.3	62.5	59.7	53.5	319.1
East	15.9	22.7	20.6	15.1	32.0	37.1	41.3	58.5	243.2
Average	16.9	19.2	20.0	17.3	44.2	47.5	54.9	55.1	825.2
Relative	115	131	136	118	300	323	374	375	-----

<sup>1</sup> L = lime.

<sup>2</sup> 1 tree; in all other cases each figure is the average of 5 or 6 trees.

TABLE 13.—*Analysis of variance for average dry weight per tree of hybrid poplars*

[Based on data in table 12]

Variation due to—	Degrees of freedom	Mean square	Observed F
Blocks	2	107.724	1.60
Treatments	15	637.341	<sup>2</sup> 10.19
Blocks X Treatments (error)	30	67.422	-----
Total	47	266.983	-----
Lime X nitrogen	1	395.027	<sup>1</sup> 5.86
Lime	1	884.942	<sup>2</sup> 13.13
Nitrogen	1	8,350.325	<sup>2</sup> 123.85
Phosphorus	1	354.797	<sup>1</sup> 5.26
Potassium	1	43.892	<1.0
First order interaction (except LN)	5	31.430	<1.0
Second and third order interactions	5	24.796	<1.0

<sup>1</sup> Significant at 5-percent level.

<sup>2</sup> Significant at 1-percent level.

Differences in leaf color were quite apparent also. The leaves of all trees receiving nitrogen were a darker green than those without it.

When the trees were removed at the end of the season it was found that the original cuttings had increased in diameter, the increase being greatest in those frames in which treatment resulted in the largest whip growth. A few representative data from this study are given in table 14. It will be recalled that all of the cuttings were of the same size when planted in the spring. The larger trees possessed longer and thicker roots also, but no measurements were made.

TABLE 14.—*Measurements showing relation between growth of whip and diameter growth of original cutting when trees were removed from frames at the end of the growing season*<sup>1</sup>

Treatment	Average dry weight of whips	Average diameter of cutting	Average weight of cutting when partially dried
	Grams	Millimeters	Grams
K	13.9	9.32	13.08
0	14.7	9.60	13.15
N	36.9	12.36	22.07
NPK	41.4	13.70	27.16

<sup>1</sup> All cuttings were the same size when planted in the spring.

#### EFFECT OF TREATMENT ON SUBSEQUENT ROOTING

Cuttings from the base, middle, and upper portions of the 18 whips held over in cold storage from the previous fall were planted in moist sand on January 31. Thirty-three days later, March 4, the shoots and roots were removed from the original cuttings and the dry weights of each obtained. Both shoot growth and root development (table 15) were largest on the base cuttings and smallest on the top cuttings. Because the previous treatments had exerted such a positive effect on the size of cuttings, it is difficult to separate the effect of treatment per se on rooting or sprouting from that of size alone. However, the ratio of both shoot and root weight to cutting weight tended to decrease with increase in size of cutting.

In the handling of the cuttings, a good many buds dropped off in spite of the care exercised to prevent it. It was observed that the cuttings which had received nitrogen the previous season lost their buds much more readily than the others. Confirmation of this behavior is necessary before conclusions can be drawn. Obviously the cuttings without buds failed to sprout or sprouted only weakly. Data from such cuttings were omitted when calculating the means in table 15.

TABLE 15.—*Average amount of shoot and root growth, and ratio of shoot and root weight to cutting weight, in relation to position on whip*

Position on whip	Average weight of—			Ratio of shoot weight to cutting weight	Ratio of root weight to cutting weight
	Cuttings	Shoots	Roots		
Top	Grams	Grams	Grams		
Top	2.11	0.44	0.025	0.211	0.0119
Middle	4.51	.63	.043	.149	.0104
Base	8.09	1.09	.074	.140	.0102

## COMPOSITION OF PETIOLES AS INDICATED BY PLANT TISSUE TESTS

The results of plant tissue tests made August 17 on the petioles from all treatments, and those of October 11 on six of the treatments are given in figures 2 and 3, together with the yield and soil test data. Figure 2 shows that the application of phosphorus definitely increased the phosphorus content of the soil in all cases, but the concentration in the petioles was increased only in those cases where growth was limited by lack of nitrogen. Potassium treatment increased the amount of available potassium in the soil; in the petioles the increases occurred chiefly on the limed plots.

Calcium was more abundant in the limed soils, of course, but the increase in the petioles was slight. Other treatments had relatively little effect on the calcium content. Magnesium in the soil was slightly greater in the limed frames but otherwise showed little variation. In the petioles magnesium was highest in the N and NP treatments; potassium appeared to repress assimilation of magnesium. Recent studies by Boynton and Burrell (1) have shown that magnesium deficiency in McIntosh apple trees may be induced by potash fertilization.

The phosphorus relationships shown in figure 3 are essentially like those in figure 2 as far as they go. However, the phosphorus content of the petioles for the PK treatment was only a fraction of what it was on August 17. Apparently, this difference is a characteristic seasonal one. The potassium concentration in the petioles was somewhat lower in October but otherwise the relationships are similar to what they were in August. Calcium and magnesium were generally higher in October. Tissue tests showed no nitrate nitrogen in the petioles.

## DISCUSSION

The relatively slight response of red pine to the treatments is not surprising considering the low requirements of that species. Norway spruce is generally considered as having somewhat higher nutritive requirements, but the limited data in this particular experiment are of little value in supporting that view. There is no question as to the response of white spruce to phosphorus, either alone or with potassium, and of the adverse effect of lime.

Among the hardwoods, southern red oak was adversely affected by lime but was apparently benefited generally by fertilizers. Sugar maple, white ash, and hybrid poplar responded readily to lime and fertilizer. The poplars as well as the spruces seemed to be adversely affected by potassium when used alone. In this connection Eriksson (5) reports that in some soils, particularly those relatively rich in sesquioxides, phosphorus fixation is increased through the application of K salts, especially in muriate form, and crop yields are reduced, except at high levels of P. Even where yields were not affected, the P content of the plant (barley and oats) was lower. Eriksson's theory is that potassium restricts hydrolysis of the amphoteric soil complexes and increases the formation and precipitation of Fe and Al phosphates. If this theory is correct it might explain the poor growth in the K-treated frames of Norway and white spruce. There is no evidence that potassium treatment had any consistent effect on the phosphorus content of the oak, maple, and ash leaves or the hybrid poplar petioles.

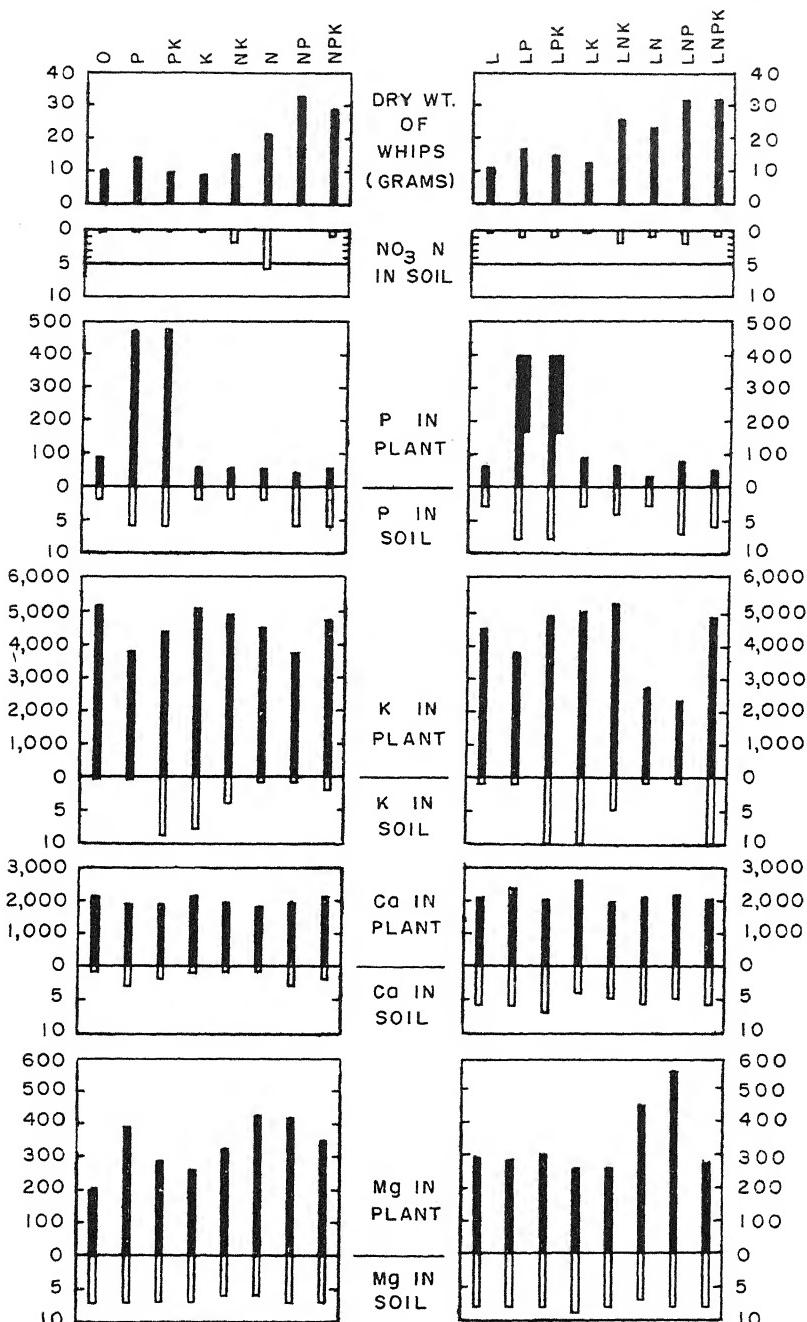


FIGURE 2.—Relation between dry weight of trees, composition of petioles, and available nutrients in soil (Morgan's quick test method). Sampled August 17. Plant petiole data given in parts per million; soil test data on arbitrary scale of 1 to 10—1 being very low and 10 very high.

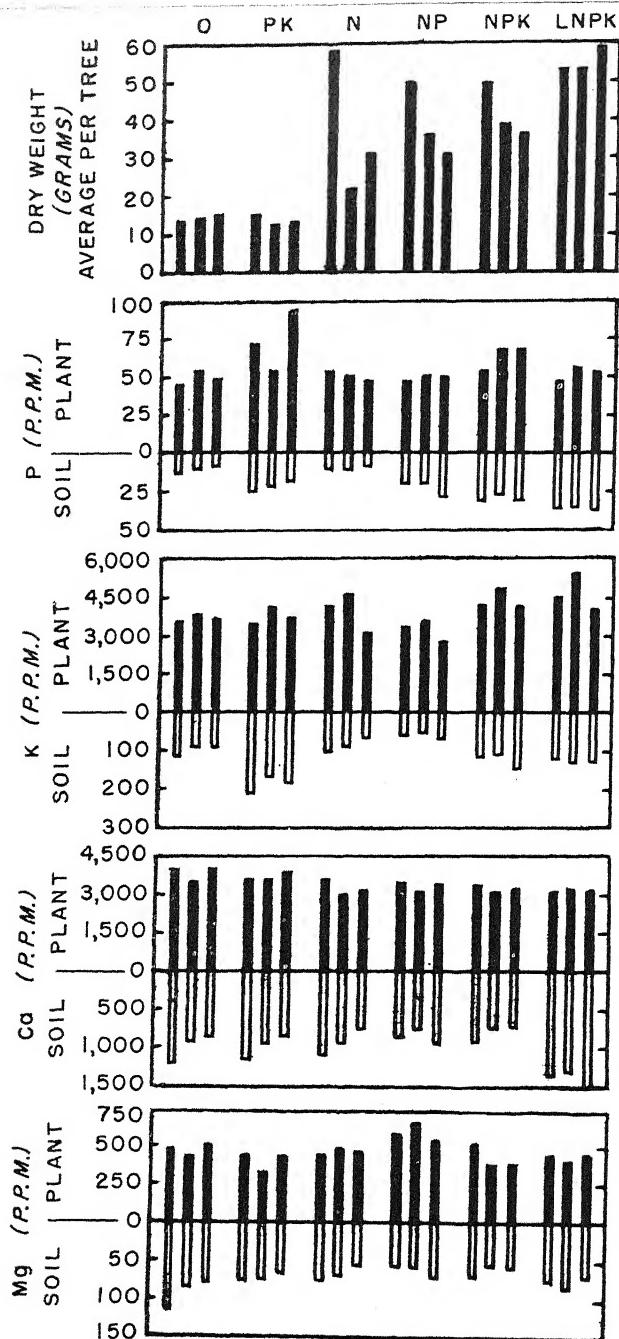


FIGURE 3.—Relation between dry weight of tree, composition of petioles, and available nutrients in the soil (Wolf method). Sampled October 11. The three bars in each treatment represent the three blocks or sets of frames.

In considering the relationships between growth, leaf or petiole composition, and soil tests, it is apparent that for most of the species tested nitrogen was the limiting element. The phosphorus content of the leaves of oak, maple, and ash increased with the increase in available phosphorus in the soil, but similar increases from potassium and calcium were evident only in the maples. The only correlation observed between growth and soil composition was in the case of red oak in which growth decreased with increase of soil calcium.

The soil under hybrid poplars contained some nitrate nitrogen in August but none in October. In August, the P and PK treatments resulted in luxury consumption of phosphorus, and there was not enough nitrogen present for satisfactory growth. The larger plants produced when P and N were included resulted in a dilution effect in the plant, although soil tests indicated an ample supply of available phosphorus. It is quite probable, however, that a still greater supply of phosphorus would have given a higher concentration of phosphorus in the petioles. This has been the experience of Scarseth (14), Carolus (2), Ulrich (19), and others, including the writer in work with table beets, spinach, and snap beans (not published). Whether or not a greater supply of phosphorus would have resulted in larger trees is not known.

Potash without nitrogen resulted in a high concentration of potassium in the soil but had only a moderate effect on the amount in the petioles. With N, however, more K was absorbed by the plant, as indicated by the somewhat lower soil test but higher plant tissue test. The available Ca content of the soil in relation to the various treatments has already been discussed (p.—). The calcium content of the petioles decreased slightly with increase in yield. The data on magnesium indicate that absorption of that element was enhanced by N and repressed by K. Süchtling et al. (18) found that liming resulted in a lower potassium content of the plant. Němec (13) found this to be true (a) where the soil was high in P and K and practically neutral in reaction, and (b) on soils low in P and K and quite acid. He found that lime increased K absorption on soils (a) high in P, low in K, and nearly neutral, and (b) high in P and quite acid, irrespective of the K content of the soil. In this study the data show very little correlation, on the whole, between available calcium in the soil and the potassium content of the leaves.

Variation between individual trees is generally greater where root space is limited and competition severe, as when five or six trees are growing in a 2-by-2 foot area. The variation exhibited by the poplars, while considerable, was much less than that shown by the preceding species which were ordinary seedlings whose genetic history was unknown. The advantages of using cuttings from a single clone are apparent.

Response to treatment by the poplars was considerably greater than it was by the previous species owing to the more rapid growth of the poplars. This rapid growth, which makes it a valuable tree in pulpwood production, as shade tree and windbreak, and for various other uses such as excelsior and cellulose manufacture (17), seems to place it in a class with farm crops in respect to nutrient requirements. There is no question but that a fast-growing tree is a more suitable indicator plant for forest trees than a fast-growing, shallow-rooted

annual like corn, for instance. Can it be assumed, however, that any fast-growing plant can indicate the needs of a slow-growing one even though they may be similar in all respects except rate of growth? For example, the requirements of oak, being low, permit the tree to live on nutrients which become available slowly in the soil—too slow for poplars. For that reason, oaks grow satisfactorily on relatively poor soil without benefit of fertilizer treatment; and if fertilizers are used, the response is only moderate. Perhaps the chief advantage of a fast-growing species as an indicator plant is to show what is needed but not how much.

While the response (or lack of it) shown by experiments of this kind are indicative of the requirements of the species in question, it is recognized that the magnitude of response observed in pot or tank cultures is not generally obtained in the field. Restriction of root space tends to intensify the differences resulting from treatment. This is particularly true with trees and other plants with large root systems. Nevertheless, the experiment shows that most species are benefited by a relatively high fertility level; that some are favored by lime and some are not, and that of the three main plant food elements, potassium is the least apt to be limiting.

Another angle of the nutrition problem—the extraordinarily favorable effect of humus materials, composts, and sod which have been reported in the literature (16)—was not touched upon in this investigation. It is, however, a profitable field for future studies in our quest for the answer to the all important question, How can the success of hardwood plantings in open fields be assured?

#### SUMMARY

A set of 48 concrete-walled frames, each 1/10,000 acre in size and containing Cheshire loam, was used to determine the growth response to lime and fertilizer of red pine, southern red oak, sugar maple, Norway spruce, white ash, and white spruce seedlings, and single clone hybrid poplar cuttings. Treatments consisted of N, P, and K, alone and in various combinations, with and without lime.

Soil tests show that the treatments resulted in very marked differences in the fertility level and acidity of the soil.

Red pine made less growth in the limed frames; differences due to other treatments were relatively small. Southern red oak was likewise adversely affected by lime. Response to other treatments was inconsistent but appeared to be greater on the nitrogen-treated soil.

Norway spruce, like red pine and oak, was adversely affected by lime.

Lime had an adverse effect also on white spruce, but P and PK were beneficial and the results were statistically significant. Root-top ratios were higher with lime than without.

The concentration of the major elements in the leaves (or petioles) of the hardwoods was increased, in general, by treatment with those elements. In oak the  $\text{SiO}_2$  content was considerably lower and the phosphorus higher on the limed soils. In maple, the ash content was affected significantly by the lime, P, and K treatments. In white ash, the phosphorus content was affected by N as well as by P; silica was influenced by lime and P; calcium by N and K; and magnesium by

lime and K. In both maple and ash, a high concentration of K in the soil resulted in a lower concentration of calcium in the leaves.

Growth of hybrid poplar was more uniform and the response to treatment considerably greater than it was with the other species. Nitrogen caused the greatest response, lime next, and phosphorus third. Potassium was inconsistent in its effect.

The original cuttings increased in size during the growing season more or less in proportion to the growth of the whips.

Subsequent rooting and sprouting of cuttings taken at the end of the season from whips produced during the summer tended to be more or less proportional to the size of the cuttings. The effect of treatment per se could not be isolated from the effect of cutting size.

In the petioles the phosphorus concentration was, in general, inversely proportional to the growth of the tree, but potassium was increased more or less irrespective of growth. Calcium increased but slightly. Magnesium absorption was highest in the NP frames and appeared to be repressed by K.

The principal facts brought out in this work are that (a) the pine, spruces, and red oak were adversely affected by lime, but sugar maple, white ash, and hybrid poplar were benefited by it; (b) response to nitrogen and phosphorus was low in the case of red pine and Norway spruce, but medium to high for all other species; (c) response to potassium, in spite of the low level of available potassium in the untreated soil, was negative in most cases. The rapid growth of hybrid poplars would seem to make them ideal for use in nutritional studies of forest trees, but the writer is of the opinion that the requirements of this or any other fast-growing tree may be quite different from those of a slow grower, hence results obtained with the former should not be applied to the latter without due caution.

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# THE EFFECT OF SOURCE OF LITTLE BLUESTEM GRASS SEED ON GROWTH, ADAPTATION, AND USE IN RE- VEGETATION SEEDINGS<sup>1</sup>

By DONALD R. CORNELIUS<sup>2</sup>

Formerly *associate agronomist, Nursery Division, Soil Conservation Service, United States Department of Agriculture*

## INTRODUCTION

The revegetation of cultivated land and depleted ranges to grass in the Great Plains during the past 10 years has required large amounts of grass seed. Under certain conditions of low rainfall and erosion, the native species were found to be better adapted than introduced species but seed supplies were not readily available. Methods of harvesting seed of grasses from native prairies have been developed, but the production of such seed in the Great Plains is erratic because of fluctuating climatic conditions, and the movement of seed supplies from other regions of the United States to meet the demands has not proved feasible.

When seed of native grasses from different sources was brought together in Soil Conservation Service nurseries to determine their relative value and adaptation for revegetation seedings, it was found that great differences existed within certain species in respect to time of flowering, seed and forage production, winter hardiness, and other factors important to the revegetation program. This situation prompted a study of little bluestem (*Andropogon scoparius* Michx.) from 16 different sources, as shown in column 2 of table 1.

## REVIEW OF LITERATURE

Variations in plant characteristics and behavior of *Andropogon furcatus* (5),<sup>3</sup> *Bouteloua curtipendula* (2, 7), *B. gracilis* (2, 3, 8, 9), *Panicum virgatum* (1), and many other species from different sources have been discussed or photographs published by several individuals. These plant variates have been designated by various names such as biotypes (2), isolates (6), geographical strains (7), and ecotypes (11, 12). The writer considers Turesson's (11, 12) designation of ecotype to be the most appropriate for the plant accessions discussed in this paper. Turesson proposed the term "ecotype" for an ecological unit

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<sup>2</sup> The author gratefully acknowledges the assistance received from members of the Kansas Agricultural Experiment Station during the course of this study.

<sup>3</sup> Italic numbers in parentheses refer to Literature Cited, p. 142.

TABLE I.—Data obtained at Manhattan, Kans., Soil Conservation Service nursery for 16 ecotypes of little bluestem (*Andropogon scoparius*) including accession number, source, flowering date (actual and theoretical), caryopsis count, dry weight per plant, and height per plant.

Accession No.	Source	Theoretical flowering date <sup>1</sup>	Actual flowering date	Caryopsis count			Dry weight of plants			Height of plants		
				1940	1941	1942	1940	1941	1942	1940	1941	1942
KG1570	Towner, N. Dak.	June 20	July 6	8.6	8.6	8.6	11.7	11.7	11.7	130	124.7	120.3
KG181	Cheyenne, Wyo.	June 21	June 11	22.0	12.6	14.0	22.1	21.6	20.7	392	28.5	25.5
KG1	O'Neill, Nebr.	July 18	July 26	20.0	11.0	24.3	18.4	21.4	406	31.0	32.6	31.7
KG588	Moorhead, Iowa	July 26	Aug. 8	15.0	9.3	23.7	16.0	18.1	455	318	31.5	38.8
KG173	El Dorado, Kans.	Aug. 17	Aug. 17	32.0	20.3	30.7	30.7	37.6	725	556	42.9	38.9
KG116	Colony, Kans.	Aug. 26	Aug. 23	32.0	26.0	22.0	26.7	35.7	588	480	43.4	52.1
KG1087	Manhattan, Kans.	Aug. 18	Aug. 19	44.7	18.3	23.0	28.7	33.7	780	589	48.9	52.5
KG380	Wyo.	Aug. 19	do	10.3	10.3	10.3	27.3	25.2	44.1	719	43.1	45.5
KG6	Guthrie, Okla.	Sept. 1	Sept. 30	22.6	20.3	26.7	27.3	407	775	580	43.1	46.1
KG1043	Orlando, Okla.	Aug. 24	Aug. 29	22.6	27.3	23.0	34.3	406	695	501	45.7	44.5
KG155	Ardmore, Okla.	Aug. 26	Sept. 8	45.0	10.3	12.7	25.7	380	551	406	52.9	47.7
KG1065	Woodward, Okla.	Aug. 13	Aug. 19	43.3	26.6	25.0	31.6	296	816	598	47.0	56.2
KG1108	Chapel Hill, N. C.	Oct. 24	Sept. 21	19.6	1.3	6.7	47.2	1.70	435	41.0	60.1	60.5
KG640	Douglas, Ariz.	July 26	Aug. 11	33.3	12.0	6.0	17.1	1.10	826	48.5	42.6	53.0
KG725	Ft. Davis, Tex.	Oct. 9	do	0	0	0	31.1	21.1	506	61.6	41.0	41.7
KG531	Vernon, Tex.	Sept. 10	Sept. 1	44.6	11.6	11.0	22.4	889	2.237	1,563	37.5	36.6
Average				31.3	15.7	17.6	21.6	346	732	540	40.9	41.5
											49.7	44.0

<sup>1</sup> Correlation of theoretical with actual flowering date;  $r=0.9819$ .

<sup>2</sup> Determined by Hopkins' biokinetic law (2).

arising as a result of the genotypical responses of an ecospecies to a particular habitat.

Although significant morphological differences were found in biotypes of *Panicum virgatum*, as reported by Nielsen (6), and *Bouteloua gracilis* and *B. curtipendula*, as reported by Fults (2), neither of these investigators found any regional segregation of races on the basis of chromosome number. The chromosome complements could not be correlated with the biotypes. Chromosome determinations were not made in this study.

#### METHODS

Seedlings were started in the greenhouse in March and transplanted to the field in May 1939. The soil was alluvial fine sandy loam of good fertility. A plant was set every 30 inches in the row and the rows were 30 inches apart. Twenty seedlings were transplanted for each ecotype, but survival was not good and only 208 plants remained for study. Complete detailed measurements were not taken for more than 10 plants of any one ecotype on account of the time required.

Since the plants did not become well established until 1940, data were not recorded in 1939. In 1940, 1941, and 1942, the seed for caryopsis counts was stripped from the plants by hand as soon as it was mature. The date that each plant came into full bloom was recorded in 1941. The dry weight of individual plants was obtained for 1940 and 1941 at the end of the growing season in October. The height of each plant was taken at the end of the growing season in 1940, 1941, and 1942.

Rod-square plots of eight ecotypes listed in table 6 were field-seeded in May 1938. One series of these plots was seeded broadcast. A second series was seeded in 14-inch rows. The soil was similar to that used for the individual plant study. A good stand emerged with both methods of seeding in early June. Observations were made until 1942.

#### EXPERIMENTAL RESULTS AND DISCUSSION

##### FLOWERING DATE

The most northern ecotypes, those from North Dakota and Wyoming, flowered in late June, whereas the more southern ones, from Texas and North Carolina, did not flower until September or October (table 1, column 3). Analysis of variance shows a highly significant difference between the time the various ecotypes came into full bloom (table 2). Statistical calculations in this paper were computed according to methods of Snedecor (10).

TABLE 2.—*Analysis of variance for flowering time of 208 little bluestem plants representing 15 ecotypes, 1941*

Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Total.....	207	188,887		
Between ecotypes.....	14	157,890	11,277.9	70.22*
Within ecotypes.....	193	30,997	160.6	

\*Exceeds F for P of 0.01 with appropriate degrees of freedom.

The actual flowering dates of the ecotypes are very close to the theoretical dates that might be expected by the application of Hopkins' bioclimatic law (4). The date, August 19, which was the actual date for the Manhattan, Kans., ecotype (accession No. KG480) was used as the base date. The latitude, longitude, and altitude of the place in which the seed originated were compared for each ecotype with similar data for Manhattan, Kans. Four days for each degree of latitude, 1 day for each degree of longitude, and 1 day for each 100 feet in altitude were added or subtracted from the Manhattan values for these three constants. The values obtained by these calculations were converted into the number of days earlier or later than the flowering date for the Manhattan ecotype (August 19) and given as the theoretical dates in column 4 of table 1. A highly significant correlation of +0.9819 was found to exist between the actual flowering date and these theoretical flowering dates based on Hopkins' bioclimatic law.

All ecotypes were observed to start growth at approximately the same time in the spring. The number of days after June 15 that each ecotype was in full flower is given in table 3.

TABLE 3.—*Average number of days after June 15 that little bluestem ecotypes from 15 different sources were in full flower at the Manhattan, Kans., Soil Conservation Service grass nursery, 1941*

Accession No.	Source	Days after June 15 (means) <sup>1</sup>
KG570	Towner, N. Dak.	4.63
KG181	Cheyenne, Wyo.	5.81
KG1	O'Neill, Nebr.	32.64
KG338	Moorhead, Iowa	39.82
KG640	Douglas, Ariz.	41.00
KG1085	Woodward, Okla.	58.94
KG1037	Manhattan, Kans.	63.50
KG473	Eldorado, Kans.	63.65
KG480	Manhattan, Kans.	65.05
KG1043	Orlando, Okla.	69.60
KG116	Colony, Kans.	71.70
KG733	Ardmore, Okla.	74.89
KG6	Guthrie, Okla.	75.79
KG531	Vernon, Tex.	87.33
KG108	Chapel Hill, N. C.	131.22

<sup>1</sup> Least significant mean difference = 10.74.

#### SEED PRODUCTION

The weight of seed produced by each plant was not determined. Caryopsis counts were made for each ecotype giving the percent of florets containing mature caryopses. These data are given in columns 5, 6, 7, and 8 of table 1. The two earliest ecotypes, from North Dakota and Wyoming, were below the yearly averages of all sources in 1941 and 1942. Early flowering frequently coincides with periods of hot winds unfavorable to seed production. Late August and early September flowering of the Kansas and Oklahoma ecotypes favor a good seed set. The late September or October flowering of the Texas and North Carolina ecotypes resulted in frost injury in 1941 and 1942. The Edna, Tex., ecotype was so late in developing inflorescences that seed was not produced in any of the 3 years.

#### DRY WEIGHT OF PLANTS

The lowest dry weight per plant was produced by the northern ecotypes from North Dakota (fig. 1, A), Wyoming, and Nebraska

(columns 9, 10, and 11, table 1). These ecotypes did not appear to grow much more than the others in early season, and they stopped growth at their flowering time about midsummer. The local (fig. 1, *B*) and southern ecotypes, on the other hand, continued growing until late summer. Some of the southern ecotypes had fresh green leaves at frost time, whereas leaves of the northern ecotypes had begun to dry by midsummer and were completely dry by late summer. The most vigorous and productive ecotype was from Vernon, Tex. (fig. 1, *C*). The nine plants ranking highest in dry weight (fig. 2) were from this ecotype. Plant breeders engaged in developing superior strains may be able to use such plants for introducing vigor and high yield. The difference between ecotypes was highly significant for each of the 2 years that weights were taken, 1940 and 1941, as shown in table 4.

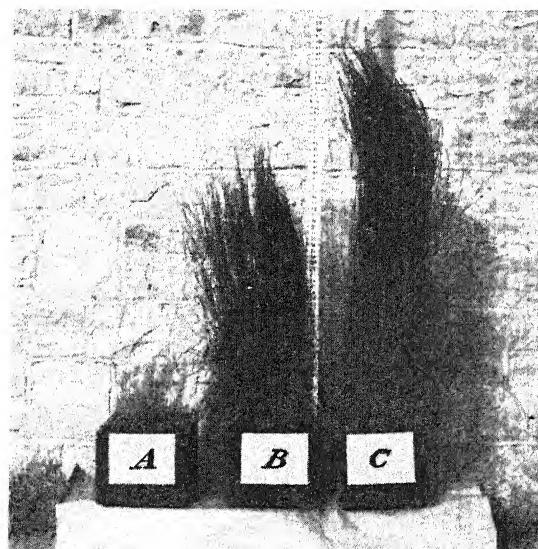


FIGURE 1.—Individual plants of little bluestem representing three different ecotypes grown at Soil Conservation Service nursery, Manhattan, Kans., 1942: *A*, KG570, Towner, N. Dak.; *B*, KG473, Eldorado, Kans.; *C*, KG531, Vernon, Tex.

TABLE 4.—*Analysis of variance of dry weight per plant for 16 ecotypes of little bluestem, 1940 and 1941*

Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Total	217	<i>1940 data</i>		
Between ecotypes	15	11,104,213	431,333	18.80*
Within ecotypes	202	4,634,224	22,942	
Total	142	<i>1941 data</i>		
Between ecotypes	15	40,136,922	1,890,331	20.38*
Within ecotypes	127	28,354,960	92,771	

\*Exceeds F for P of 0.01 with appropriate degrees of freedom.

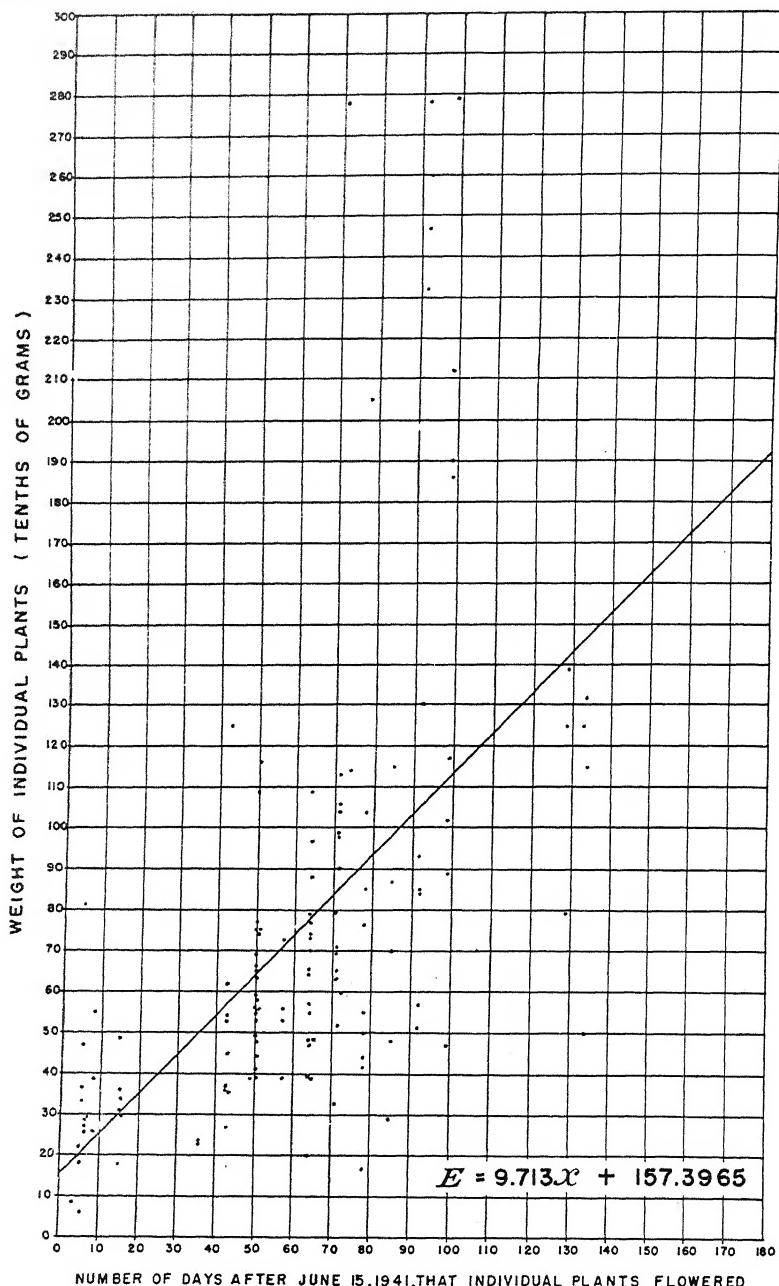


FIGURE 2.—Regression of dry weight at maturity on flowering date in 133 plants of little bluestem grown at the Manhattan, Kans., Soil Conservation Service nursery in 1941.

## HEIGHT OF PLANT

The North Dakota and Wyoming ecotypes averaged less than 30 inches for the 3-year period (table 1). The Nebraska and Iowa ecotypes were about 35 inches tall, whereas those from Kansas and Oklahoma ranged from an average of 44.7 to 53.0 inches for the 3 years. The greater altitude of Douglas, Ariz., apparently produced an effect similar to northerliness, as was shown by the fact that the Douglas ecotype had a height of only 37.6 inches, flowered earlier, and had a lower dry weight than other southern ecotypes. The Vernon, Tex., ecotype was the tallest, the average height being 61.3 inches, with one plant in 1942 having the maximum height of 78 inches. The relative height of northern, local, and southern ecotypes is shown in figure 1. The difference in height of the various ecotypes was found to be highly significant by analysis of variance (table 5).

TABLE 5.—*Analysis of variance for height of 16 ecotypes of little bluestem, 1940 to 1942, inclusive*

Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Total	219	<i>1940 data</i> 17,971		
Between ecotypes	15	13,249	883.27	38.15*
Within ecotypes	204	4,722	23.15	
Total	214	<i>1941 data</i> 24,656		
Between ecotypes	15	18,658	1,244	41.47*
Within ecotypes	199	5,998	30	
Total	211	<i>1942 data</i> 26,738		
Between ecotypes	15	21,857	1,457	58.28*
Within ecotypes	196	4,881	25	

\*Exceeds F for P of 0.01 with appropriate degrees of freedom.

## FLOWERING DATE AND WEIGHT OF PLANT

Flowering time usually marks the end of vigorous growth for most grasses. Therefore, early flowering of warm season grasses reduces the length of time in which the plants produce the best grazing and lowers the total yield of dry weight per plant in plants left to the end of the growing season. To determine the effect of flowering date upon dry weight of plant at maturity the regression coefficient was determined for 133 plants in 1941 as  $E=9.713X+157.3965$ , as given in figure 2. Correlation of these two characters was +0.5775, which is highly significant for the appropriate degrees of freedom.

## WINTER HARDINESS

The individual plants in this test were sufficiently favored by location to permit all the ecotypes to survive the winter. The susceptibility of certain ecotypes to winter injury was found when different ecotypes were compared in rod-square plots. Unfortunately, all of the same ecotypes could not be tested in both rod-square plots and as individual plants because of limited seed supply and facilities. The

rod-square plots did not afford enough replications for statistical analysis. However, the differences between certain ecotypes were sufficiently great, as shown in figures 3 and 4, to justify the conclusion that all ecotypes should be carefully tested for winter survival before they are moved very far northward for large scale plantings. Table 6 gives the percent of winter injury for eight ecotypes tested in 1940-41.

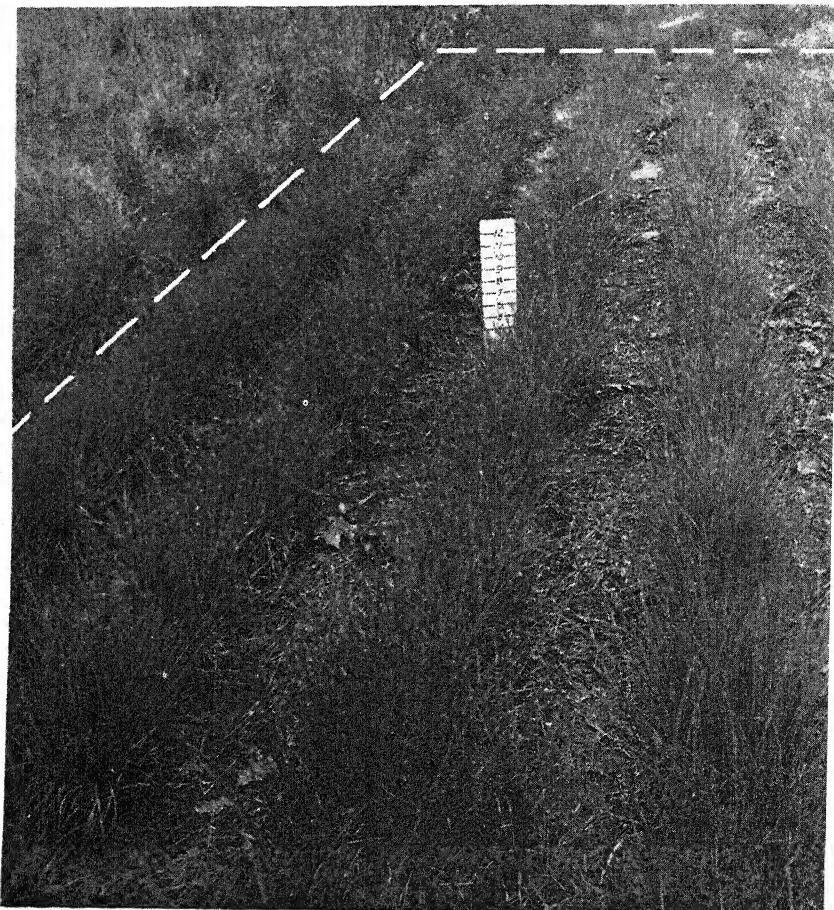


FIGURE 3.—Little bluestem ecotype, origin Manhattan, Kans., grown in rod-square plot Soil Conservation Service nursery at Manhattan, showing good spring growth as a result of complete winter hardiness. Photographed May 10, 1941.

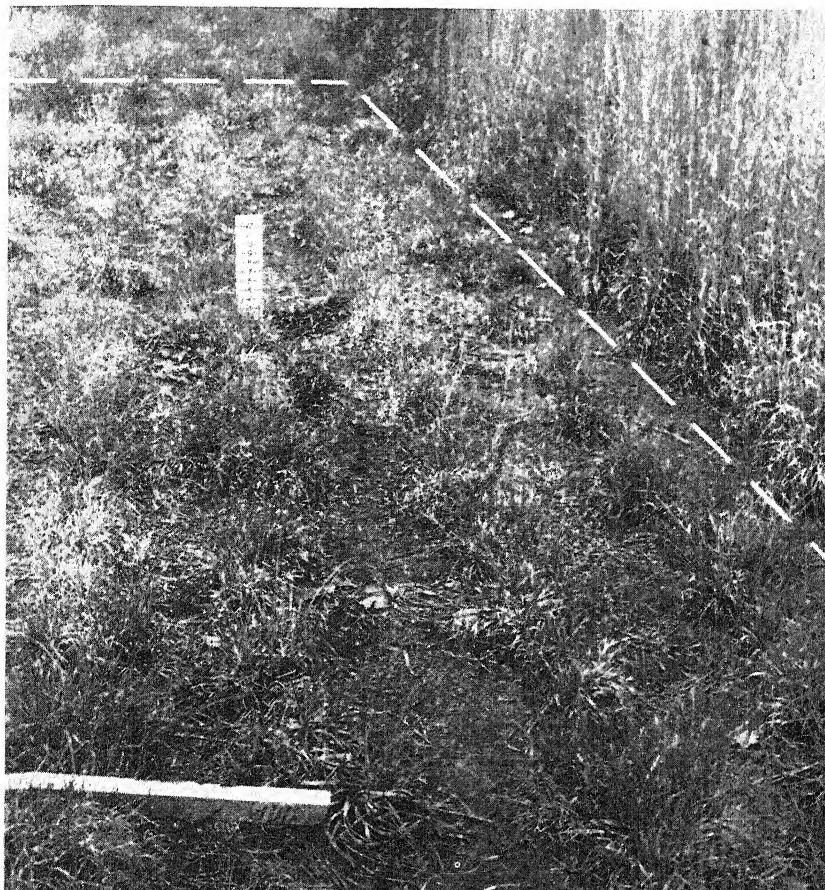


FIGURE 4.—Little bluestem ecotype, origin Schulenburg, Tex., grown in rod-square plot Soil Conservation Service nursery, Manhattan, Kans., showing weak spring growth and considerable winter injury. Photographed May 10, 1941.

TABLE 6.—*Winter injury of little bluestem ecotypes tested at Manhattan, Kans., Soil Conservation Service nursery in rod-square plots, 1940-41*

Accession number	Origin of ecotype	Percentage injury	
		Broad-cast	14-inch rows
KG743	Towner, N. Dak.	0	0
KG732	O'Neill, Nebr.	0	0
KG480	Manhattan, Kans.	0	0
KG478	Colony, Kans.	0	0
KG763	Idabel, Okla.	20	30
KG740	Clovis, N. Mex.	50	10
KG750	Gatesville, Tex.	80	40
KG737	Schulenburg, Tex.	75	70

## SUMMARY

Little bluestem (*Andropogon scoparius*) plants were grown at the Soil Conservation Service nursery, Manhattan, Kans., from seed obtained from 16 different sources representing different ecotypes. Two series of rod-square plots in which eight ecotypes were represented were observed from 1938 to 1942.

The northern ecotypes were earlier in maturity and lower in forage production than the southern ones. The local ecotypes were intermediate. Higher seed set was obtained with the local ecotypes, which flowered in late August or September. The early northern ones suffered the hot winds of midsummer at flowering time and the later southern ones were sometimes frosted in October.

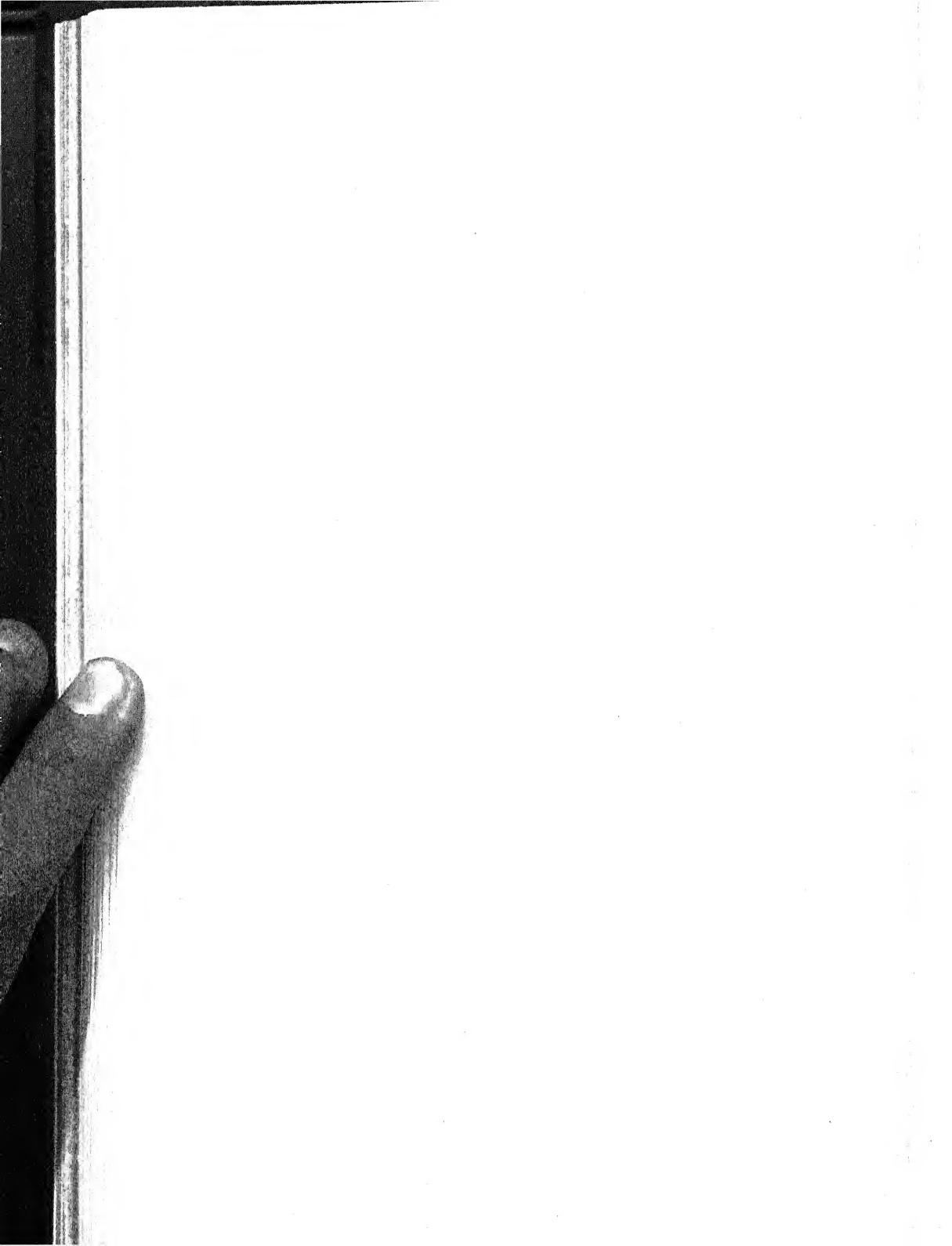
There were significant differences between ecotypes with regard to time of flowering, dry weight at end of growing season, and height. A high correlation existed between actual flowering date and a theoretical date computed from Hopkins' bioclimatic law which takes into consideration the difference in latitude, longitude, and altitude of the original sources. A highly significant correlation was found between flowering date and dry weight of plant at close of season. The regression coefficient for the effect of flowering date on dry weight of plant was determined.

Winter injury of southern ecotypes was observed in rod-square plots in which eight ecotypes were tested. It appears to be advisable to test ecotypes carefully before seed is transported great distances for large-scale plantings.

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## BLACKENING INDICES OF POTATOES GROWN UNDER VARIOUS CONDITIONS OF FIELD CULTURE<sup>1</sup>

By W. E. TOTTINGHAM, late *associate professor of biochemistry*; RUDOLPH NAGY,  
A. FRANK ROSS, JERRY W. MAREK, and CARL O. CLAGETT, former *research assistants in biochemistry*, Wisconsin Agricultural Experiment Station

### INTRODUCTION

In an earlier paper<sup>2</sup> the writers presented results from greenhouse cultures which indicated that the variety and relative instability of the seed stock are primary factors contributing to the darkening of boiled potatoes (*Solanum tuberosum*). The results described here were obtained with field plots on Antigo silt loam (Antigo, Wis.), Plainfield sandy loam (Hancock), and Miami silt loam (Madison). Hot, dry weather was characteristic of the summers during the early years of the study, and extensive areas produced tubers which blackened on cooking.

### GENERAL PROCEDURE

Unless otherwise specified, certified seed stocks were used. Plots were 1 rod square on silt loam and 1 by 2 rods on the other soils. The fertilizer treatments were randomized by plots with replication on adjacent sections of the field. Application of the fertilizer (400 pounds per acre on sandy loams and 800 pounds on silt loams) was made by hand from a prepared portion of each plot. The fertilizer was placed in the hill, covered by soil, and the tuber was planted above it. Except limited trials of irrigation, the remaining care of the crop followed customary cultural practice.

A storage temperature for tubers of 60° F. was used prior to, and 40° F. during and after the 1939 trials. The experience of the writers indicates that the tendency to darken on cooking appears after about 2 months' storage at 40° F. and after about 1 month at 60°. The procedure for judging and expressing discoloration has been described.<sup>3</sup> Briefly, it consists of boiling 0.25-inch thick longitudinal slices from 10 tubers, cooling them by exposure to air, and comparing their discoloration with photographic standards. Tubers were scored as 0=white,

<sup>1</sup> Received for publication December 15, 1945. Supported in part by grants from the Wisconsin Alumni Research Foundation.

<sup>2</sup> TOTTINGHAM, W. E., NAGY, R., ROSS, A. F., and others. A PRIMARY CAUSE OF DARKENING IN BOILED POTATOES AS REVEALED BY GREENHOUSE CULTURES. *Jour. Agr. Res.* 67: 177-193, illus. 1943.

<sup>3</sup> RIEMAN, G. H., TOTTINGHAM, W. E., and McFARLANE, J. S. POTATO VARIETIES IN RELATION TO BLACKENING AFTER COOKING. *Jour. Agr. Res.* 69: 21-31, illus. 1944. See also footnote 2.

1=light gray, 2=medium gray, and 3=dark gray. The scores for the 10 tubers were added and the sum divided by 0.3; thus if 10 tubers each scored 3 their sum of 30 divided by 0.3 would give the maximum blackening index of 100; a set of all medium gray tubers would have a blackening index of 66 $\frac{2}{3}$ , etc.

#### FIELD TRIALS

##### TESTS OF 1933

The test at Madison, Wis., in 1933 was designed to determine the effects of a liberal application of potassium to the soil and of irrigation on darkening of the cooked product. The complete fertilizer was a 2-10-12.5 mixture compounded from commercial sodium nitrate, 16 percent acid phosphate, and potassium sulfate. Four applications of water each lasting a half hour were made to each plot with a rotary lawn sprinkler. Canvas covers were applied to other plots to shed rain. The weather was very hot and dry in June ( $8.8^{\circ}$  F. above and 2.1 inches below normal), hot in September ( $4.8^{\circ}$  above normal), but otherwise essentially normal.

Tubers of the Irish Cobbler variety from plots which had been unfertilized or to which no potassium had been applied, cooked slightly gray after 2 weeks' storage, but all Rural New Yorker tubers boiled white. Longer storage might have changed the response somewhat.

##### TESTS OF 1934

The same plots were planted at Madison in 1934 as in 1933 but with a reversal of the controlled water relations. Other plantings<sup>4</sup> were made as follows: At Antigo; at Spooner in northwestern Wisconsin; at Almond near Hancock; at Tunnel City on sandy loam in western Wisconsin; and at Racine on Clyde silt loam in southwestern Wisconsin. A badly discoloring Rural New Yorker seed stock obtained from Tunnel City was used for comparison with a normal one. The fertilizer was similar to that used in 1933, but half the nitrogen was carried in cottonseed meal.

The weather record for Almond is taken from observations at Hancock and is so classed in figure 1. Spooner was wet in September (2.9 inches above normal) and dry in July (1.7 inches below normal). It was warm in June ( $3.0^{\circ}$  F. above normal) and cold in September ( $3.4^{\circ}$  below normal). At Tunnel City (Viroqua records) the precipitation alternated from wet to dry in successive months. This area was hot in early summer ( $8.6^{\circ}$  and  $5.0^{\circ}$  above normal in June and July, respectively). Racine was wet in September (3 inches above normal) and dry earlier (average deficit of 1.6 inches in July and August). It was hot in June and July ( $5.0^{\circ}$  and  $2.6^{\circ}$  above normal) and cool in September ( $2.7^{\circ}$  below normal). These records together with those in figure 1 and table 1 show that the 1934 crop was subjected to heat at all locations during early vegetative activity but matured in cool weather. At Madison, Racine, and Spooner it was subject to drought until the generally heavy precipitation of September. This may have resulted in seriously reduced availability of mineral nutrients.

<sup>4</sup>The writers are indebted to Dr. J. C. Walker and Prof. J. G. Milward of the experiment station staff and to G. A. Johnson, a local producer, for care of the plots at Racine, Spooner, and Tunnel City, respectively.

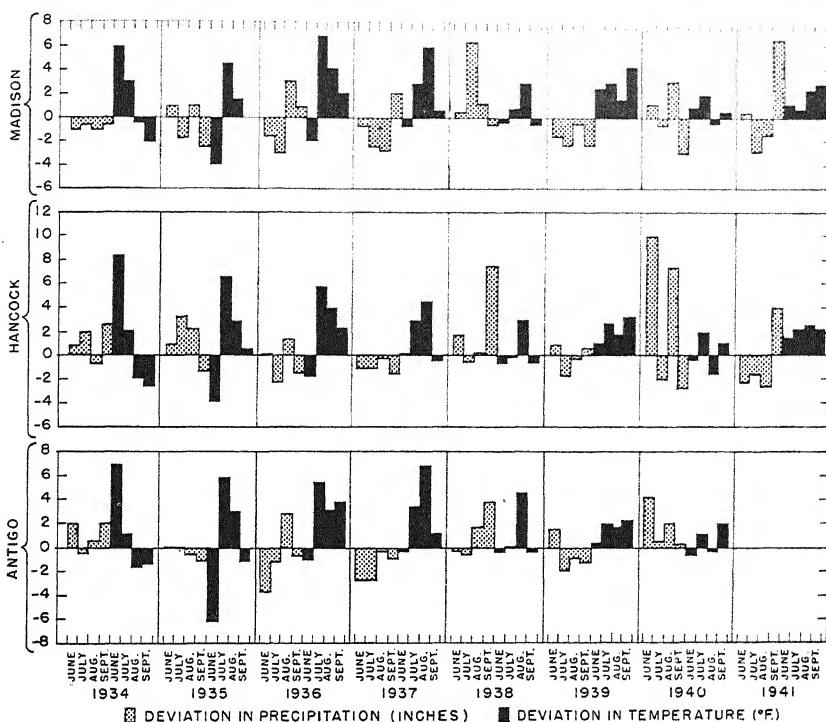


FIGURE 1.—Deviation in temperature ( $^{\circ}$ F.) and precipitation (inches) from the normal mean values for the months of June, July, August, and September for the years 1934 to 1941, at the Madison, Hancock, and Antigo stations.

One-half of a sample of tubers taken at Almond about September 1 had peaked ends, while those from Antigo were mildly misshapen. The Almond crop from both seed stocks that received no potash cooked white. The crop from normal seed stock unfertilized at Antigo cooked light gray, and the crop from discoloring seed stock that received complete fertilizer became medium gray. Otherwise this crop cooked white regardless of fertilizer supply and irrigation. Immaturity of potatoes apparently is not a direct cause of blackening after cooking, for the immature tubers in this test showed no uniform blackening.

Boiling responses of the matured potatoes are shown in table 2. Blackening occurred independently of fertilizer treatment and irrigation and did not follow the discoloration response of the seed stock. With the exception of early heat the weather was relatively favorable,

TABLE 1.—Normal mean temperature and precipitation values for the growing season of 1934 at the Madison, Hancock, and Antigo stations

Station	June		July		August		September	
	Temper- ature	Precipi- tation	Temper- ature	Precipi- tation	Temper- ature	Precipi- tation	Temper- ature	Precipi- tation
Madison . . . . .	67.2	3.76	71.8	3.88	70.0	3.21	60.0	3.72
Hancock . . . . .	65.3	4.47	69.9	3.45	68.9	3.41	57.3	3.81
Antigo . . . . .	63.0	4.38	66.6	4.1	65.7	3.23	56.3	4.03

and on the whole the darkening was rather mild. The Burbank variety at Madison scored light gray with and dark gray without added potash.

TABLE 2.—*Blackening index of mature Rural New Yorker tubers, 1934*

Location	Weeks after harvest	Normal seed stock				Discoloring seed stock			
		Unfer- tilized	No potash	Com- plete fertilizer	Com- plete fertilizer and irrigation	Unfer- tilized	No potash	Com- plete fertilizer	Com- plete fertilizer and ir- rigation
Almond	2		0	0			0	0	0
Antigo	2	33	0	0	33	0	33	67	0
Madison	2		0	0		0	0	33	33
Racine	3	67		0		0	0	33	
Spooner	3	0		0		0	0	33	
Tunnel City	20	0		0		0	0	0	

In 1934 other members of the Wisconsin station staff began to provide the writers with potatoes produced under different conditions of fertilization and irrigation. A. R. Albert, superintendent of the branch station at Hancock, furnished tubers of the Rural New Yorker variety grown at Almond, to which single materials (Mn, Mg, Ca, and S) had been applied. These tubers were white to dark gray on boiling without consistent relation either to the particular element applied or to changes in soil acidity.

Prof. F. L. Musbach, of the Department of Soils, supplied samples of Rural New Yorker crops produced on Kennan silt loam at Ashland in the extreme northern part of Wisconsin. Application of either manure alone (10 tons per acre) or manure (7.5 tons per acre) and 500 pounds of 3-10-6, 3-10-10, or 3-10-20 fertilizer led to white-cooking tubers throughout; the same results were obtained on Plainfield sandy loam at Spooner. Other tubers from Ashland produced on a second crop of clover supplemented by 800 pounds of 3-12-12 or 3-12-18 fertilizer showed slight bluish-gray discoloration on boiling. The weather at Ashland (Bayfield records) was only slightly cool and dry during the period from June to September (total deficit of 3.0 inches and monthly average of 1.3° F. below normal). However, August was distinctly cool (3.3° below normal).

W. B. Ogden and Dr. James Johnson provided samples of Rural New Yorker potatoes produced on Miami silt loam at Madison. The plots had received lime and 10 tons of manure per acre before 3 successive years of cropping to tobacco. In the year preceding potatoes the crops had been legumes, timothy, tobacco, or a cereal grain. Eleven potato crops from such plots cooked white 3 weeks after harvesting.

#### TESTS OF 1935

The 1935 plots were located at Antigo (in another portion of the field planted in 1935 but still subject to previous overliming), at Arnott, and on both peat and loam at Madison. Examination of the surface soil sampled at planting<sup>5</sup> gave the results shown in table 3.

<sup>5</sup>For the soil analyses reported in this paper the writers are indebted to Dr. H. H. Hull of the Department of Soils of the University of Wisconsin.

The peat had been fertilized annually for corn production; a 3-11-16 fertilizer was applied for the potato test. Variations in practice included withholding either one-half of the nitrogen or one-third of the potash until tubers had begun to form.

The weather records show that precipitation was much greater than normal in July at Arnott (Stevens Point records), and continued so into the period of tuber development. September was dry, especially at Madison. The temperature at all locations shifted from excessively low to excessively high during vegetative activity. September was moderately cool at Antigo (Wausau records).

TABLE 3.—*Partial record of available nutrients in soils at planting, 1935*

Location	Phosphorus <sup>1</sup>	Potash <sup>1</sup>	Nitrate nitrogen <sup>2</sup>	pH
Antigo.....	90	175	20	6.1
Arnott.....	70	200	Trace	5.3
Madison, loam.....	125	350	33	6.0
Madison, peat.....	150	275	65	6.5

<sup>1</sup> Pounds per acre to a depth of 8 inches.

<sup>2</sup> Parts per million.

The boiling records given in table 4 were taken during the period from September 26 to December 1. Double columns represent replicate plots. Serious discoloration was confined to the Antigo crop and to susceptible varieties. Low potassium was at times associated with blackening; however, complete fertilizer did not prevent the trouble at Antigo. In general the results indicate some correlation of discolora-

TABLE 4.—*Blackening index of potatoes in relation to seed stock and fertilizer, 1935*

Location	Seed stock	Fertilizer application <sup>1</sup>				
		None	No potash	Complete	Late potash	Late nitrogen
Antigo.....	Burbank.....	33	67 67	67 0	0 0	0 0
	Cobbler.....	0	0 0	0 0	0 0	33 0
	Katahdin.....	0	33 0	0 0	0 0	0 0
	Rural New Yorker, normal.....	33	0 0	0 0	0 0	0 33
	Rural New Yorker, discoloring .....	0	33 33	67 0	0 0	0 0
	Burbank.....	0	33 0	0 0	0 0	0 0
Arnott.....	Cobbler.....	0	0 0	0 0	0 0	0 0
	Katahdin.....	0	0 0	0 0	0 0	0 0
	Rural New Yorker, normal.....	0	0 0	0 0	0 0	0 0
	Rural New Yorker, discoloring .....	0	33 33	0 0	0 33	0 0
	Burbank.....	20	33	33	33	33
	Cobbler.....	20	0	0	0	33
Madison, on loam .....	Katahdin.....	20	0	0	33	0
	Rural New Yorker, normal.....	20	0	0	0	0
	Rural New Yorker, discoloring .....	20	33	0	0	0
	Burbank.....	0	0	0	0	0
	Cobbler.....	0	0	0	0	0
	Katahdin.....	0	0	0	0	0
Madison, on peat .....	Rural New Yorker, normal.....	0	0	0	0	0
	Rural New Yorker, discoloring .....	0	0	0	0	0
	Burbank.....	0	0	0	0	0
	Cobbler.....	0	0	0	0	0
	Katahdin.....	33	33	0	0	0
	Rural New Yorker, normal.....	0	0	0	0	0
Spooner.....	Rural New Yorker, discoloring .....	0	0	0	0	0

<sup>1</sup> Double columns represent replicate plots.

<sup>2</sup> Complete fertilizer with irrigation.

tion with propensity of the seed stock and with fertilizer treatments, so into the period of tuber development. September was dry, especially at Madison. The temperature at all locations shifted from excessively low to excessively high during vegetative activity. September was moderately cool at Antigo (Wausau records).

Other samples produced under normal cultural conditions in northern Wisconsin were provided by Dr. J. C. Walker. Of these, the Chippewa, Cobbler, Katahdin, and Warba varieties were relatively white after boiling, whereas the Beauty of Hebron, Russet Burbank, and Rural New Yorker became appreciably gray.

#### TESTS OF 1936

In 1936 the field work at Antigo was transferred to another farm<sup>6</sup> in order to avoid soil conditions conducive to scab. In central Wisconsin the work was transferred to the branch agricultural experiment station at Hancock, where cultural operations were supervised by Prof. A. R. Albert. Other potato varieties were introduced and more extensive randomizing<sup>7</sup> of fertilizer treatment by plots and varieties in each plot was applied. Soil samples were taken at times of active assimilation of mineral nutrients by the crop.

The normal application of fertilizer on clay loam was 670 pounds per acre of 6-10-20, with nitrogen and potassium added as sulfates. Potassium was varied to half and double the normal amount, and on some plots the low potassium fertilizer was supplemented with farm manure (8.4 and 4.2 tons per acre on clay loam and sandy loam, respectively) or its equivalent of nitrogen as ammonium sulfate. In other cases the manure was supplemented by hydrated lime (1,670 pounds on loam, 835 pounds per acre on sand) or one-half its equivalent of nonalkaline "lime" as hydrated calcium chloride. Certain plots at Madison received the usual irrigation four times from July 7 to August 2.

Data for soil samples taken at planting were essentially the same as in table 3 (Hancock substituted for Arnott) except that values of 45 and 5 for phosphorus and nitrate at Antigo and 20 for phosphorus at Hancock should be substituted. Data covering available potash at harvesting appear in table 5. Availability remains better in the sandy loam than in the clay loams, suggesting potassium fixation in the clay loams during the dry season. The irrigated plots at Madison

TABLE 5.—*Soil content of available potash at harvesting in 1936*

Location	Unfertilized	No. K <sub>2</sub> O	Low K <sub>2</sub> O	Medium K <sub>2</sub> O	High K <sub>2</sub> O	Low K <sub>2</sub> O with—		High K <sub>2</sub> O with—	
						Manure	Ammonium sulfate	Lime hydrate	Lime chloride
Antigo.....	150	140	225	200	210	175	150	190	150
Hancock.....	125	175	200	225	340	260	140	225	250
Madison.....	275	275	-----	300	-----	-----	-----	325	325

<sup>1</sup> No K<sub>2</sub>O.

<sup>6</sup> The writers are indebted to the owner, A. O. Reznichek, for constant interest in the project and faithful care of the crop.

<sup>7</sup> With the aid of Dr. J. H. Torrie, Department of Agronomy.

TABLE 6.—*Blackening index of potatoes produced in field plots at different locations, 1936*

Location and date of test	Variety	Fertilizer treatment					
		Unfertilized	No K <sub>2</sub> O	Low K <sub>2</sub> O	Medium K <sub>2</sub> O	High K <sub>2</sub> O	Medium K <sub>2</sub> O + manure
Antigo; Oct. 23-24	Burbank.....	67	67	67	33	0	33
	Chippewa.....	0	0	0	0	0	0
	Cobbler.....	0	0	0	0	0	0
	Green Mountain.....	33	33	0	0	0	33
	Rural New Yorker.....	0	33	67	33	0	0
	Triumph.....	0	0	0	33	67	67
Hancock; Oct. 20-23	Burbank.....	33	67	33	0	0	33
	Chippewa.....	0	0	0	33	0	0
	Cobbler.....	0	0	33	33	0	0
	Rural New Yorker.....	67	67	33	67	33	67
	Triumph.....	0	33	0	0	0	0
	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> only						
Madison; Nov. 1-3	Burbank.....	0	0	0	0	0	33
	Chippewa.....	0	0	0	0	0	0
	Cobbler.....	0	0	0	0	0	0
	Rural New Yorker.....	0	0	0	0	0	0
	Triumph.....	0	0	0	0	0	0
	Manure only						
Spooner; Dec. 10	Burbank.....	33	33	33	33	33	33
	Chippewa.....	0	0	0	0	0	0
	Cobbler.....	33	33	33	33	33	33
	Rural New Yorker.....	33	0	0	0	0	0
	Triumph.....	0	0	0	0	0	0
	Ca(OH) <sub>2</sub> only						

to which complete and potash-free fertilizer had been added were found to contain, respectively, 800 and 300 pounds of available potash per acre at harvesting.

At the Spooner branch experiment station duplicate plots were planted under the supervision of Professor Milward. A rare mineral mixture supplied 25 pounds of manganese salt, 10 pounds of copper salt, 5 pounds each of boron and zinc salts, and 1 pound each of salts of bromine, cobalt, iodine, molybdenum, selenium, titanium, and vanadium.

From figure 1 it is apparent that rainfall was seriously deficient during the main period of vegetative activity and became excessive in August. The temperature was more extreme, being much above normal after June. It seems probable that the prolonged heat was more conducive to blackening of the tubers than was the early drought.

The discoloration record is shown in table 6. Crops produced at Madison were characteristically free of blackening. In accord with their usual stability, the Chippewa and Triumph varieties were almost universally white on cooking, whereas the Burbank and Rural New Yorker discolored at all locations but Madison. The Cobbler was relatively free of blackening except in the Spooner crops; this may possibly be ascribed to difference in seed stocks. The Green Mountain produced at Antigo discolored mildly. The rarer elements did not prevent blackening of the crop at Spooner.

There is little correlation between the discoloration and omission of potassium from the fertilizer. Medium levels of potash appeared best, for the complete omission of fertilizer or more liberal applications of potash were more frequently followed by blackening. In the latter case it appears possible that higher concentrations of salts may have been injurious in a dry period.

After storage at 40° to 50° F. until the latter half of March the unfertilized and potash-deprived Burbank crops from Antigo and the Rural New Yorker crops from Madison cooked medium gray. In other cases these stocks had improved; the Antigo Rural New Yorker which had been supplied lime salts became white.

#### TESTS OF 1937

Primary attention was given in 1937 to potassium availability and to the application of rarer mineral elements. New soil areas were occupied at Antigo, Hancock, and Spooner. At Spooner each plot consisted of two rows 68 feet long following clover sod. The general fertilizer was a 3.5-9-18 mixture. Supplementary "x" and "y" salt mixtures were added as specified in table 7. The "x" mixture supplied per acre 6.2 pounds manganese, 2.5 pounds copper, 0.9 pound boron, and 1.1 pounds of zinc. The "y" mixture contained equal weights of salts of bromine, cobalt, iodine, molybdenum, selenium, titanium, and vanadium. Zinc was included in the "y" mixture applied at Antigo. Agrico carried rarer elements in unspecified forms and amounts. The application of ferrous sulfate carried 10 pounds of iron per acre. Examination of the soils from Antigo and Hancock before planting showed equal contents of available potash (155 pounds per acre) and ammonia nitrogen (35 pounds per acre) and similar acidity (pH 5.3 and 5.2, respectively). The available phosphorus and nitrate nitrogen

TABLE 7.—*Blackening index of potatoes in relation to seed stock and fertilizer, 1937*

Location	Fertilizer	Variety					
		Burbank	Chippewa	Cobbler	Green Mountain	Rural New Yorker	Triumph
Antigo	{ None	33	33	0	33	33	0
	No potash	33	33	33	33	33	67
	Low potash	0	0	0	33	33	0
	High potash	33	0	0	0	33	0
	Agrico	33	0	33	67	67	0
	Complete +B Cu	33	33	67	33	33	0
	Complete +B Mn	33	0	33	67	67	0
	Complete +B Cu Mn	0	0	0	67	67	0
	Complete +Cu Mn	0	0	0	33	67	0
	Complete +x+y <sup>1</sup>	67	0	33	0	33	0
Hancock	Complete +Fe	0	0	0	33	33	33
	Complete +Fe Cu	33	0	33	67	67	0
	Katahdin						White Gold
	None		0		33	67	67
Madison	Complete	33		67	33	67	33
	Complete +x	67	0		33	67	33
	Complete +x+y	33	33	33	0	67	67
	Triumph						
Spooner	{ None	33	33	33		0	33
	No potash	0	0	0		0	33
	Irrigation, potash	33	67			33	33
	Complete	0	0	0		33	0
	Irrigation, potash			33		0	33
Spooner	Complete +x	0	0			67	33
	Complete +x+y	0	0			33	33
	Green Mountain						
	No potash	33	0	67	67	67	33
Spooner	Complete	33	33	67	67	33	0
	Complete +x+y	33	33	67	33	67	0
	Agrico	33	33	33	67	67	33

<sup>1</sup> For composition of x and y mixtures see p. 152.

of the Antigo soil (55 pounds per acre each) were about three and five times, respectively, the amount in the Hancock soil.

At the time of harvesting, samples of soil were taken at the four locations. These showed no consistent relations between available nutrients and fertilizer applications. The Antigo soil had become more alkaline (pH 5.5). The values were 5.4 for Spooner and 6.0 for Madison. Available phosphorus had increased in the Antigo soil to the same level as in the Madison soil (125 pounds per acre). At Hancock and Spooner it was double the original level at Hancock, or 50 pounds per acre. Available nitrogen was unchanged at Antigo but the ammoniacal form was four times that at Madison. Hancock had been depleted of available nitrogen to 5 pounds per acre of each form, but the similar soil at Spooner contained 40 pounds of nitrate and 15 pounds of ammonia nitrogen per acre. The data for potash were inconsistent in regard to increases with applications, but the average levels at Hancock and Spooner (about 170 pounds per acre) were little more than one-half those at the other locations. Samples of the subsoil taken at this time did not show a downward accumulation of available potash. It therefore seems probable that applied potash not assimilated by the crop was leached from the sandy soils and fixed by colloidal clay in the silt loams.

The Burbank stock was apparently a healthy one. It had been produced on peat soil at Coddington, Wis., in 1936 from stock grown in Idaho. The Cobbler stock was obtained from Prince Edward Island in 1935 and had been rogued at Antigo in 1936. The Rural New Yorker stock was provided by Professor Albert; the writers' plots were operated in conjunction with his at Hancock.

The principal boiling records are assembled in table 7. These display in the Antigo crops the notable records of the Chippewa and Triumph varieties for whiteness after boiling. The Green Mountain and Rural New Yorker varieties, and to a lesser extent the Burbank and Cobbler varieties, display their common susceptibility to blackening. Unfavorable regional effects are evident at Hancock with the Rural New Yorker variety and at Spooner with the Rural New Yorker and Green Mountain varieties. The 1936 tests at Madison with iron, boron, and copper supplements and with irrigation showed more serious darkening of the Chippewa and less darkening of the Triumph variety than in the 1937 tests. The Cobbler and Rural New Yorker varieties from Madison blackened frequently.

Extensive tests of single fertilizer constituents, and various levels and proportions of the major elements were conducted at Hancock in collaboration with Professor Albert. The Rural New Yorker used was light to medium gray after boiling; apparently the effects of heat and drought were dominant over the fertilization factors in the production of tuber pigment. However, it appears that high soil temperature alone was not the primary cause of the discoloration, for Dr. R. H. Larson repeatedly obtained relatively high records (over 90° F.) at a depth of 3 inches at Madison between mid-July and early September. Discoloration was not as serious in Madison, and corresponding soil temperatures did not induce blackening of greenhouse crops.

Professor Albert supplied samples from experiments dealing with the influence of different proportions of legume crop ploughed under and the application of farm manure. Tubers of the Katahdin variety produced on land from which only one crop of alfalfa had been removed in 1936 cooked light gray as compared with medium gray of a crop following alfalfa sod after two cuttings. This favorable effect of the larger crop residue was obtained from both unfertilized land and from land that had received 500 pounds per acre of 0-9-27. Rural New Yorker tubers from land manured after 6 years of cropping to alfalfa cooked light gray. These results seem to indicate a beneficial effect from maintaining the organic matter in the soil.

Other potato samples were supplied by Professor Musbach. The Green Mountain variety produced at Antigo under irrigation, either unfertilized or with the application of 600 and 2,400 pounds per acre each of 3-12-0 and 3-12-18, cooked white or light gray. Without irrigation, the crops produced without fertilizer and those produced by the use of 600 pounds of 3-12-0 cooked medium gray, but where 600 pounds of 3-12-18 was applied the discoloration was slight. The Rural New Yorker produced at Ashland by the addition of 7.5 tons per acre of manure or the same with 500 pounds of either 0-10-10 or 3-10-20 cooked light gray. So also did the crop on second growth of clover with 800 pounds per acre of 3-12-12.

Rural New Yorker tubers produced at Spooner after application of

7.5 tons of manure per acre cooked medium gray, but supplementary use of 500 pounds of commercial fertilizer containing different quantities of nitrogen and potash was followed by improvement to light gray. Land on which there had been second growth of clover plus 800 pounds of commercial fertilizer produced tubers which cooked light gray, even when the fertilizer contained 18 percent of potash.

Other samples, provided by J. L. Garrard, field agronomist of the American Potash Institute, were produced on peat at Coddington in central Wisconsin. Tubers of the Cobbler variety were essentially white after boiling, whether unfertilized or supplied 300 pounds per acre of nitrogen-free fertilizer. The fertilizer contained various combinations of phosphoric acid and potash, ranging from 0 to 20 and 0 to 50 respectively. The Rural New Yorker produced on Plainfield sandy loam with application of 300 pounds of 3-12-12 fertilizer and irrigation cooked medium gray. An unirrigated portion of the crop otherwise similarly treated remained acceptably white. Other samples became light gray, regardless of either omission or 50 percent increase of the potash supply and irrespective of 25 percent increase or decrease of phosphorus supply.

As figure 1 shows there was a marked deficiency of precipitation at Antigo during the vegetative period of June and July and a moderate deficiency during late tuberization in September. However, the most striking climatic departure was excessive heat during August. The high temperatures from July to September furnished a climatic hazard which might have induced the serious discoloration found at Antigo.

Hancock was subject to a mild deficiency of rainfall throughout the season. The weather was hot in July and especially in August. Although not as abnormal in this respect as at Antigo, the heat was probably more critical on the light sandy soil at Hancock. Tuber discoloration was most serious in the crop of this region.

Madison suffered severely from both heat and drought in July and August, and the crop showed practically as much discoloration as at Antigo.

Spooner, with a soil type essentially the same as that at Hancock, had a similar record of precipitation except a rainy July. Its temperature record was very similar to that of Hancock save for an excessively hot August. Except for Chippewa, the varieties grown at Spooner had the better boiling record, which might be expected from its cooler northern location.

Samples of potatoes produced by commercial growers near Antigo were made available to the writers. Cobbler and Rural New Yorker varieties were taken from fields of Felix Zeloski late in September. Immediately after harvesting, the Cobbler variety cooked white regardless of irrigation. The Rural New Yorker from an unirrigated portion of the field also cooked satisfactorily white, but the irrigated crop cooked light gray. The Rural New Yorker produced by L. D. Sargent with application of 3-9-16 fertilizer cooked fairly white where irrigated once, but light gray where irrigated four times. These results suggest that the effect of irrigation is to increase discoloration in boiled potatoes.

Different varieties of potatoes showed a distinct difference in blackening index when grown under the conditions of excessive heat and drought characteristic of 1937.

## TESTS OF 1938

The planting at Antigo was on two of the replicate sections used in 1937. The "complete" fertilizer was a 3-10-18 mixture carrying nitrogen and potassium as sulfates. Elements of the x mixture were supplied in essentially the usual forms and amounts, with the exception of 10 pounds of boric acid per acre. Plots with straw mulch were added. The plots at Hancock were located on an area previously in general cropping. Three replications were made of the treatments shown in table 8. At Madison the plot tests were transferred to a sandy slope on the university campus. The higher and lighter soil of this area was devoted to a comparison of varieties, while the more clayey portion was used for application of various fertilizer combinations to the Rural New Yorker variety.

TABLE 8.—*Blackening index of potatoes in relation to variety and fertilizer, 1938*

Location	Fertilizer	Variety 12			
		Chippewa	Cobbler	Rural New Yorker	Triumph
Antigo	None	0	33	33	33
	No potash	0	0	33	33
	Low potash	33	33	33	33
	High potash	0	0	33	33
	Rarer elements	0	33	33	33
	Rarer elements—boron	0	0	33	33
	Complete+rarer elements	0	33	33	33
	Rarer elements—boron	0	0	33	33
	High potash, mulched	0	0	33	33
	(None)	Ospr <sup>2</sup>	0	Ospr	33spr 33spr
Hancock	No potash	0	0	0	33spr 33spr
	Low potash	0	0	0	33spr 67spr
	High potash	Ospr	0	0	33spr 33spr
	Rarer elements	Ospr	0	Ospr	33spr 67spr
	Rarer elements—boron	Ospr	0	Ospr	33spr 33spr
	Complete+rarer elements	Ospr	0	Ospr	33spr 33spr
Madison	Complete—boron	0	0	Ospr	33spr 67spr
	Complete+rarer elements	0	0	0	33
	(Complete+rarer elements)	0	33	33	0

<sup>1</sup> Double columns represent replicate plots.

<sup>2</sup> Spr indicates occurrence of "spraing" or internal brown spotting.

At Hancock light "spraing" or internal brown spotting appeared to be of universal occurrence in the raw tubers of Rural New Yorker and of frequent occurrence in the Chippewa and Cobbler varieties. The evidence from the latter varieties indicates that spraing and blackening after boiling are not directly associated. Greenhouse cultures on sand had earlier shown a frequent occurrence of spraing when the moisture content of the sand was low. Freedom from blackening of the Cobbler crop at Hancock contrasts with its record of previous seasons, suggesting that the seed stock may have been unusually stable. The universal freedom of the Triumph variety from discoloration is characteristic of that variety.

Further cultural treatment of the Rural New Yorker at Hancock included the x mixture of rarer minerals (Mn, B, Cu, and Zn), together with molybdenum and cobalt, and the same without boron, applied both alone and in combination with a "complete" fertilizer. In some cases the K<sub>2</sub>O was omitted from such mixtures. Tubers from all of these treatments cooked light gray, even when irrigated liberally.

During the vegetative period rainfall (fig. 1) was somewhat excessive at Hancock and Madison, and during September it was high at Hancock and Antigo. So far as temperature departures are concerned, the only serious abnormalities seem to be the high temperatures in August.

Cobbler and Chippewa tubers produced at Antigo blackened after boiling; those produced at Hancock cooked normally. Discoloration of the Chippewa variety is rare. The Triumph retained its record for whiteness of cooking at all three locations. Inherited varietal differences appear to be a major factor in tuber discoloration.<sup>8</sup>

Samples of Rural New Yorker tubers produced at Ashland on Superior clay loam were supplied by Professor Musbach. The tubers produced on the lower levels of potash with manure (no fertilizer or 3-10-6) and those following clover cooked medium gray. Those following manure supplemented by higher levels of potash cooked light gray and darkened only mildly at the stem ends. The Rural New Yorker was also produced on Plainfield sandy loam at Spooner. Only the lower levels of potash (no fertilizer and 3-10-6) were associated with medium grayness of the cooked tubers, all of the others becoming light gray. The Triumph variety was also produced at Spooner; borax was added alone (10 pounds) and at different levels (5, 10, 20 pounds) in combination with 600 pounds of 3-12-12 fertilizer. The crops that were supplied the low borax mixture, both irrigated and unirrigated, and those supplied the borax-free mixtures unirrigated, cooked light gray while all the others remained white. This would seem to show a correlation between discoloration and boron deficiency, but such an interpretation is not supported by the total experience of the writers.

The major climatic abnormality at both Ashland and Spooner was excessive heat in August. In addition, the latter location suffered a deficiency of rainfall until August. It appears probable that of the climatic factors that might have influenced the results, heat was primarily responsible for inducing darkening of the susceptible Rural New Yorker variety in both locations.

#### TESTS OF 1939

The primary object of the 1939 test was to determine the effect on discoloration of tubers of different levels of boron supplied in conjunction with different levels of potassium in a "complete" fertilizer. A highly purified muriate of potash (KCl) low in boron was used. Phosphorus was supplied in calcium metaphosphate (61 percent available  $P_2O_5$ ) and nitrogen as ammonium sulfate. Five, 10, or 30 pounds of borax were added to an 800-pound-per-acre quantity of "complete" fertilizer. Also, 40 pounds of borax was applied as a supplement with 25 pounds of manganese salt. As a more complete treatment this formula was supplemented by 5 pounds each of copper and zinc salts together with 1 pound each of salts of cobalt, iodine, molybdenum, selenium, thallium, titanium, and vanadium. At Hancock, on sandy soil, fertilizers were applied at one-half of these rates. These plots were relocated on new areas at Antigo and Hancock but not at Madison. In the Madison area the rate of application of fertilizer was increased to 1,200 pounds per acre.

<sup>8</sup> See footnote 3, p. 145.

A partial record of the composition of these soils in the midst of cropping is shown in table 9. At Antigo a liberal supply of potassium was followed by a sustained abundance of this element in the soil, but the results at Hancock were somewhat erratic. There was a notable increase of nitrate with increase of potassium supply at both Antigo and Hancock.

TABLE 9.—*Partial composition of soils in early August 1939*

Location	Available P	Available K <sub>2</sub> O	N as NO <sub>3</sub>	N as NH <sub>3</sub>	pH
Antigo:					
Low K	80	160	20	40	5.5
Medium K	60	170	25	45	5.2
High K+high B	95	280	35	40	5.3
Hancock:					
Low K	105	180	25	30	5.2
Medium K	80	170	35	25	5.0
High K	65	260	40	25	4.9
High K+low B	60	160	50	30	5.0
Madison:					
Sandy area, east	200	400	60	40	7.0
Sandy area, west	160	400	75	45	6.3
Loamy area, east	100	200	50	35	7.0
Loamy area, west	220	450	75	40	6.3

The plots at Madison were deficient in rainfall in both June and July; the other stations were deficient in July only. In August and September rainfall was deficient at both Antigo and Madison. This was a season of prolonged but mild heat in which Antigo, Hancock, and Madison suffered increasingly in the order named.

Records of the color of the potatoes after boiling are assembled in table 10. These are general averages, largely of 2 samples from different plots at Madison and of 3 samples from the other locations. The total record of the Chippewa variety was 9 light gray and 30 white samples at Antigo, 5 slight to light gray and 30 white at Hancock. Thus, the discoloration after cooking was at least no greater in the sandy area at Hancock than farther north. It is worthy of note that most of the cases of spraing occurred independently of darkening after boiling.

TABLE 10.—*Blackening index of potatoes produced in field plots, 1939*

Location and date of test	Treatment	Chip-pewa	Cob-bler	Rural New Yorker; normal	Rural New Yorker; discoloring A	Rural New Yorker; discoloring B
Antigo: (Boiled Nov. 27-Dec. 5; average of 3 plots.)	Low K	11	33	33	22	22
	Low K+low B	0	22	33	33	44
	Low K+medium B	0	33	33	22	33
	Low K+high B	16	44	33	22	44
	Medium K	11	44	33	22	22
	Medium K+low B	0	22	11	22	33
	Medium K+medium B	33	44	22	11	33
	Medium K+high B	0	33	22	44	33
	High K	11	44	33	11	22
	High K+low B	0	33	22	16	22
	High K+medium B	0	33	22	22	44
	High K+high B	11	33	22	33	44
	High K+high B+Mn	22	44	33	22	33
	High K+high B+x+y	0	33	22	22	33

† For composition of x and y mixtures see p. 152.

TABLE 10.—*Blackening index of potatoes produced in field plots, 1939—Continued*

Location and date of test	Treatment	Chip-dewa	Cobbler	Rural New Yorker, normal	Rural New Yorker; dis-coloring A	Rural New Yorker; Dis-coloring B
Hancock:	Low K	0	30	0	—	33
(Boiled Dec. 7-13; average of 3 plots.)	Low K+low B	0	22	22	33	0
	Low K+medium B	0	22	11	0	0
	Low K+high B	11	11	22	0	11
	Medium K	0	22	33	0	33
	Medium K+low B	0	0	33	0	33
	Medium K+medium B	0	0	33	33	33
	Medium K+high B	11	11	16	0	33
	High K	0	0	33	0	22
	High K+low B	0	22	33	0	33
	High K+medium B	0	22	11	—	50
	High K+high B	0	22	16	16	0
	High K+B+Mn	11	11	11	—	—
	High K+high B+x+y	0	22	44	0	—
	Complete	0	16	9	—	0
	Complete+x	0	0	0	—	0
	Unfertilized	—	—	6	—	—
Madison:	x only	—	—	0	—	—
(Boiled Nov. 13-14; Rural New Yorker section; average of 2 plots.)	x+Co, Mo	—	—	0	—	—
	x+B	—	—	0	—	—
	Complete high K	—	—	0	—	—
	Complete High K+x	—	—	16	—	—
	Complete low K	—	—	0	—	—
	Complete low K+x	—	—	0	—	—

The Cobbler produced at Antigo included 8 samples that boiled medium gray, 27 light gray, and only 3 that showed no discoloration. This record was much worse than that at Hancock, which included 17 light-gray samples, 19 white, and 15 cases of light spraing. Of 3 stocks of Rural New Yorker planted at Antigo and Hancock, 2 produced tubers that turned light gray after boiling. These formed the source of the discoloring seed stocks A and B shown in table 9. From the progeny of normal Rural New Yorker stock there were 34 samples that boiled slight to light gray and 8 that boiled white at Antigo; at Hancock there were 1 medium gray, 20 light gray, and 10 white. Discoloring Rural New Yorker seed stock A yielded 26 light-gray samples and 10 white ones at Antigo, as compared with 13 white and 3 light-gray at Hancock. Discoloring Rural New Yorker seed stock B produced 3 medium-gray samples, 36 light gray, and 4 white at Antigo, as compared with 2 medium gray, 21 light gray, and 4 white at Hancock. The general record of discoloration was more serious throughout in the Antigo crop; spraing and dark stem ends were confined to the Hancock crop. As usual, the Madison crop was notable for its whiteness without regard to the seed stock planted. The weather records indicate that the season was somewhat hot and dry but not excessively so and the weather abnormalities were about the same at the three stations (fig. 1). On the whole, there was no great difference in the response of the normal and discoloring Rural New Yorker seed stocks at Antigo; the discoloring stock A gave the best results at Hancock. No correlation was found between boron supplied and the degree of darkening after cooking.

## TESTS OF 1940

The plantings at Antigo and Hancock were on land not recently used for potatoes. Six plots were treated with a low potassium fertilizer (6-12-6) and six with a high potassium fertilizer (6-12-18). Ten seed stocks were planted, the order being randomized for each pair of adjacent low and high potash plots. This arrangement gave a triplicate test of each fertilizer formula. Five of the stocks had cooked acceptably white and five had turned appreciably dark. The stocks used were predominantly from the Rural New Yorker variety.

Antigo and Hancock had a considerable excess of rainfall during both the early vegetative and the early tuberizing periods of the crop. Except for possible early removal of nutrients from the soil at Antigo, these departures seem likely to have favored rather than interfered with the plants. At Hancock, however, the great excess of precipitation during these periods must surely have led to serious depletion of fertility in the sandy soil. The departures of temperature from normal were probably too small to cause any disturbance in plant development.

The results from a determination of available mineral nutrients in soil samples taken the first week in October appear in table 11. These show an accumulation of potassium in all cases following the higher application of potassium. A native deficiency of phosphorus in the sandy soil at Hancock seems to have been substantially offset by the fertilizer application. The usual predominance of nitrate over the ammoniacal form of nitrogen is here largely reversed in the Miami silt loam. Dr. K. C. Berger, of the Department of Soils, who determined available boron in soils from areas planted at Antigo, Hancock, and Madison from 1937 to 1939, found overlapping values at the different locations in the range 0.2-0.5 p. p. m., so that it does not appear that deficiency of this element is associated with the tuber discoloration in question.

TABLE 11.—*Available nutrients in soil at the close of the cropping season, 1940*

Location	Available P	Available K <sub>2</sub> O	N as NO <sub>3</sub>	N as NH <sub>3</sub>	pH
Antigo:					
Unfertilized divider rows.....	100	145	Trace	20	5.2
Composite low-K plots.....	100	150	Trace	20	4.8
Composite high-K plots.....	85	210	5	20	4.8
Hancock:					
Unfertilized divider rows.....	30	140	Trace	20	5.0
Composite low-K plots.....	50	150	Trace	15	4.9
Composite high-K plots.....	110	220	15	20	4.9
Madison:					
East (sandier) side of plots.....	110	310	40	10	6.4
West (more loamy) side of plots.....	110	446	30	5	6.4

The boiling records of the crops described above are shown in table 12 as the average of three plots per seed stock, except for single plots at Madison. Discoloration was erratic. Seed stock No. 5 was Triumph, No. 7 was Cobbler, and the others were of the Rural New Yorker variety. Although the normal seed stocks showed a better record than discolored seed stocks on low potassium fertilization, this was not true on high potassium. Tubers from the discolored seed stocks were not appreciably inferior, despite the fact that this group had a Cobbler (No. 7) to balance the white-cooking Triumph (No. 5) of the normal group. The more liberal supply of potassium seems to

TABLE 12.—*Blackening index of potatoes<sup>1</sup> produced in field plots, 1940*

Location	K level of fertilizer	Normal seed stocks					Discoloring seed stocks				
		1	2	3	4	5	6	7	8	9	10
Antigo	Low K	33	22	33	44	0	33	33	56	33	33
	High K	33	22	11	11	0	0	33	11	11	11
Hancock	Low K	22	11	22	22	0	44	22	33	33	22
	High K	33	44	33	33	11	33	0	22	22	33
Madison	High K	0	0	0	0		0		0	0	0

<sup>1</sup> Seed stock No. 5 was the Triumph variety, No. 7 was Cobbler, and the rest were Rural New Yorker.

## TESTS OF 1941

be associated with the production of more normal tubers, except at Hancock, with normal seed stocks.

Because of the negative results in previous years of attempts to control blackening after boiling by the application of fertilizer, plot operations were restricted in 1941. At Hancock two plots were treated with 3-10-12 fertilizer supplemented by the x mixture of minor elements, and a third plot was treated with the same fertilizer except that the percentage of nitrogen was doubled. The potassium chloride salt was low in boron.

At Madison, on the sandy area, one section was unfertilized and another was treated with the fertilizer supplemented by minor elements, as applied at Hancock. In addition to the certified stocks planted here, stocks which discolored after cooking but appeared otherwise normal were separated into tubers normal and abnormal on the basis of cooking tests with individual tuber sections ("indexed" tubers, table 13). These were planted in separate sections of a row fertilized as described above.

Other plantings were made on Miami silt loam at the university farm adjacent to the campus. These were on small plots (0.0055 acre) which received low, medium, and high applications of KCl (125, 250, and 500 pounds per acre respectively), with unfertilized check plots. Relatively high levels of calcium hydroxide and calcium sulfate (gypsum) at the rate of 1,000 pounds each per acre, were applied both without and with potassium in proportions indicated with the data. The rows were placed 6 feet apart, with a distance of 2 feet between hills.

Hancock suffered from a considerable deficiency of rainfall except during tuberization of late varieties (fig. 1). Madison had an equal

TABLE 13.—*Blackening index of potatoes produced in field plots, 1941*

Location and treatment	Chippewa	Cobbler	Rural New Yorker	Indexed Chippewa		Indexed Rural New Yorker	
				White	Gray	White	Gray
Hancock:							
Unfertilized	0	0	33				
Normal fertilizer	0	0	33	0	33	33	66
High-N fertilizer	0	0	33	0	0	33	33
Madison:	0	0	33				
Unfertilized	0	0	0	0	0		
Normal fertilizer	0	0	0	0	0		

deficit during the period July-August, but the type of soil at this location was more tolerant of drought. At both locations the crop was subjected to temperatures considerably above normal.

The boiling records are assembled in table 13. As usual, the crops at Madison were notably free from discoloration. At Hancock the Rural New Yorker variety was distinguished from the other varieties by its susceptibility to blackening. The comparison of normal with discolored seed stocks shows some indication of a carry-over of this response to the succeeding crop.

#### RESULTS WITH SELECTED SEED STOCKS

For the plantings of 1939 seed stocks of Rural New Yorker and a few other varieties were obtained from a number of growers in Wisconsin. In addition, stocks of different varieties were obtained from potato specialists in other States. The object of this phase of the work was to determine whether the cooking response of the crop was maintained under different climatic and soil environments. Uniform fertilizer and cultural treatments were applied throughout for these crops on portions of the field plots at Antigo, Hancock, and Madison.

The records of crops produced from these stocks in 1939 are shown in table 14. The tubers produced at Hancock discolored somewhat more than those from Antigo. The tubers from Madison cooked white.

TABLE 14.—*Blackening index of crops produced from selected seed stocks at different locations, 1939*

Source of seed stock	Variety and stock	Boiling record of seed stock	Boiling record of crop at—		
			Antigo	Hancock	Madison
Colorado:					
A.	Rural New Yorker	0	33	67	—
B.	Burbank	67	0	67	—
B.	Rural New Yorker	67	33	67	0
Idaho:					
A.	British Queen	33	33	33	—
A.	Burbank	33	33	0	—
A.	Idaho Russet	33	0	33	—
Maine:					
A.	Rural New Yorker	0	33	53	0
B.	Chippewa	0	0	—	—
B.	Cobbler	33	0	—	—
B.	Rural New Yorker	33	0	—	—
Minnesota: B.	Red Warba	0	0	0	—
Nebraska:					
A.	Chippewa	0	0	0	—
A.	Cobbler	0	67	33	—
A.	Triumph	0	0	—	—
B.	Chippewa	0	0	—	—
B.	Cobbler	0	33	—	—
B.	Triumph	0	0	—	—
New York: A.	Rural New Yorker	67	33	33	0
Wisconsin:					
A.	Early Ohio	0	0	—	—
A.	Early Rose	0	0	33	0
A.	Red McClure	0	33	67	—
A.	Discoloring (indexed Rural New Yorker)	67	67	67	—
B.	Sprained (selected Rural New Yorker)	0	33	33	0
A.	Seabago	0	—	—	0
C.	Rural New Yorker	33	0	67	0
D.	do	33	33	33	0
A.	Triumph	0	0	0	0
E.	Rural New Yorker	0	0	67	—
F.	do	0	33	33	—

The response of the crop was not consistently related to the boiling record of the stock. Six of the 28 stocks planted at Antigo produced crops showing increased discoloration and 7 stocks showed the reverse relation. Eight of 20 stocks planted at Hancock produced crops showing increased discoloration and only 2 produced less discoloring crops. Thus about 50 percent of the stocks at each station showed no change. At Antigo the effect of the change of environment was indefinite and at Hancock it was unfavorable to whiteness of the cooked crop. The few stocks tested at Madison showed a favorable effect of location upon the boiled tubers.

The crops produced from these seed stocks at Antigo in 1939 were used as planting stock for a repetition of the test in 1940. Results of the boiling tests are assembled in table 15.

TABLE 15.—*Blackening index of crops produced from selected seed stocks at different locations, 1940*

Source of seed stock	Variety	Boiling record of seed stock	Boiling record of crop at--		
			Antigo	Hancock	Madison
<b>Colorado:</b>					
A	Rural New Yorker.....	0	0	-----	0
B <sub>1</sub> .....	Burbank.....	67	0	0	0
B <sub>2</sub> .....	Rural New Yorker.....	67	0	-----	0
<b>Maine:</b>					
A.....	Rural New Yorker.....	0	0	33	0
B <sub>1</sub> .....	Cobbler.....	0	-----	-----	0
B <sub>2</sub> .....	Green Mountain.....	-----	-----	-----	0
B <sub>3</sub> .....	Rural New Yorker.....	67	0	0	-----
<b>Minnesota:</b>					
Nebraska:	Red Warba.....	0	0	0	0
<b>Nebraska:</b>					
A <sub>1</sub> .....	Cobbler.....	0	0	33	-----
A <sub>2</sub> .....	Triumph.....	0	0	0	-----
B <sub>1</sub> .....	Cobbler.....	0	-----	-----	0
B <sub>2</sub> .....	Triumph.....	0	0	0	-----
B <sub>3</sub> .....	Chippewa.....	0	-----	-----	0
<b>New York:</b>					
Wisconsin:	Rural New Yorker.....	67	0	67	0
<b>Wisconsin:</b>					
A <sub>1</sub> .....	do.....	33	-----	67	0
B <sub>7</sub> .....	do.....	33	33	33	0
D <sub>10</sub> .....	do.....	0	0	33	-----
E <sub>26</sub> .....	do.....	0	0	-----	-----
Antigo.....	Triumph.....	0	0	0	0
<b>Hancock:</b>					
	Rural New Yorker.....	0	0	-----	-----
	Cobbler.....	0	0	0	-----
	Triumph.....	0	0	0	-----
<b>Madison:</b>					
	Rural New Yorker.....	0	0	0	0
	Chippewa.....	0	0	0	0

None of the crops produced at Antigo showed greater discoloration than the original seed stock. Five showed less discoloration. About 80 percent showed no change. Approximately one-half of the crop produced at Hancock discolored no more than the original stock, about one-sixth discolored less, and one-third discolored more. Conditions at Hancock again induced discoloration of unstable varieties. All of the 14 stocks tested at Madison cooked white, which is in accord with the results of the test in 1939. The 1940 season was less conducive to discoloration than that of 1939 (fig. 1).

#### DISCUSSION AND SUMMARY

The tests reported here covering field trials over a period of 9 years, were designed to ascertain the influence of climatic and cultural conditions and potato varieties on the blackening of potatoes after boiling. By extending the trials over a number of years at several stations a variety of soil types and climatic conditions were encountered, and it

was possible to test numerous potato varieties and strains and to employ a number of different fertilizer treatments.

These extensive tests show no single factor or combination of factors to be uniformly responsible for blackening of potatoes on boiling; rather, they emphasize the complexity of the factors governing this response of tubers.

Diverse fertilizer treatments on different soils, including the use of both the macro- and micro-nutrient elements, gave little consistent alleviation of blackening in the tubers grown. The only fertilizer element that exerted any marked control of blackening was potassium. Frequently a high level of potassium decreased blackening noticeably, but this response was not uniform even within an experiment. To attribute any specific action to potassium or any other nutrient element in controlling blackening is unjustified by data thus far accumulated.

That climatic or soil conditions are involved in blackening was strongly suggested by the fact that potatoes grown at Madison consistently boiled whiter than those produced at other locations. In the absence of substantial evidence that mineral nutrition governs blackening of tubers, climatic factors are implicated. Judging by the yearly incidence of blackening in market potatoes, one would conclude that the trouble was associated with growth in a hot, dry season. However, the hottest and driest station among those reported is Madison, and there the least blackening occurred. In contrast to the general observation that hot, dry seasons induce a tendency to blacken, Smith, Nash, and Dittman<sup>9</sup> found that little or no blackening occurred in potatoes which matured when the mean temperatures were 70° F. or higher, but potatoes which matured when the mean temperatures were 60° or lower often blackened. Blackening could be prevented by exposing susceptible tubers to 100° for 3 to 4 days. Consistent with the view of Smith et al., Madison had the highest mean temperature during the maturation period and the lowest incidence of blackening. Climatic factors evidently influence the tendency to blacken, but the data are not sufficiently consistent to permit a precise definition of the conditions that cause it.

Variety more directly than any other factor governs the blackening of potatoes.<sup>10</sup> It is rare that Chippewa, Triumph, or Sebago potatoes cook black regardless of the conditions under which they are produced; in contrast, Rural New Yorker, Irish Cobbler, and some other varieties often cook black.

Fertilization with the macro- and micro-nutrient elements does not insure the production of potatoes that will remain white on boiling, and approach to the problem from this direction currently holds little promise. Climatic conditions evidently are involved, but the governing factors are ill-defined, and aside from control of water supply by irrigation they cannot be altered appreciably. On the other hand, a rather consistent blackening or nonblackening response is predictable on the basis of tuber varieties. In the present state of our knowledge, probably the best assurance of producing nonblackening potatoes rests in the selection of proper varieties, e. g., Triumph, Chippewa, and Sebago, which regularly yield acceptable tubers.

<sup>9</sup> SMITH, O., NASH, L. B., and DITTMAN, A. L. POTATO QUALITY VI. RELATION OF TEMPERATURE AND OTHER FACTORS TO BLACKENING OF BOILED POTATOES.—Amer. Potato Jour. 19: 229-254. 1942.

<sup>10</sup> See footnote 3, p. 145.

## PREDISPOSITION OF TOMATO TO FUSARIUM WILT<sup>1</sup>

By R. E. FOSTER, formerly *research assistant*, and J. C. WALKER, *professor of plant pathology*, Wisconsin Agricultural Experiment Station<sup>2</sup>

### INTRODUCTION

Fusarium wilt (*Fusarium oxysporum f. lycopersici* (Sacc.) S. & H.) of tomato (*Lycopersicon esculentum* Mill.) is a representative of the vascular fusarial diseases. In this group of maladies, the pathogen, after gaining entrance into the plant through the root system, becomes established in the xylem where toxic materials are produced which pass throughout the plant, bringing about yellowing and wilting of the leaves and stem and later causing death. Two rather distinct phases of host development enter into the complex which determines its susceptibility or resistance to the pathogen. One of these is the effect of the xylem contents on growth of the pathogen and the production of toxic materials. The other is the anatomical and physiological make-up of the plant which determines the degree to which it resists or succumbs to the pathogen.

Observations made during the course of previous studies (24)<sup>3</sup> led the authors to believe that wilt development may be affected not only by environmental factors existing during infection and disease development, in which case the first phase mentioned above is primarily concerned, but also by factors acting upon the plants before infection and in some manner altering their susceptibility or resistance, in which case the second phase may be expected to be the one involved. It was indicated that control of environmental factors acting upon the host alone for a period of time before infection might bring about differences in subsequent disease development distinct from the influence of such factors acting upon the host-pathogen complex after infection. The concept is one which has received little study in the general field of the relation of environment to disease development.

Previous studies have nearly always included the effect on disease development of factors in the environment acting before, during, and after infection of the host. It is, therefore, difficult to determine to what extent those factors were effective in predisposing the host to infection and disease development. Dickson (6) found in the case of seedling blights of corn and wheat (*Gibberella saubinetii* (Mont.) Sacc.) that soil temperatures influenced the susceptibility of the

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<sup>2</sup>The writers wish to express their appreciation to Mr. Eugene Herrling for preparation of the illustrations.

<sup>3</sup>Italic numbers in parentheses refer to Literature Cited, p. 184.

hosts to disease. Wheat seedlings were more severely infected when grown at high soil temperatures, while corn seedlings succumbed more rapidly when grown at low soil temperatures. Dickson and Holbert (8) showed that marked changes in the metabolism of corn seedlings occurred at the same temperature at which susceptibility to blight was increased. Shaw (19) found that both soil and air temperatures before infection affected the susceptibility of apple shoots to fire blight (*Erwinia amylovora* (Burr.) Bergey et al.). A number of workers (7, 18, 19, 23) have noted that the water content of the soil affected the host in such a way as to render it more or less susceptible to disease. The effect of nutrition on the development of host-parasite relations has received considerable attention, but only limited studies on the relation between host nutrition and predisposition to disease have been reported (5, 11, 13, 18, 19, 22). That a given plant may be more susceptible to disease at one stage of development than at another is a well recognized fact. According to Blank (2), older cotton seedlings showed a greater susceptibility to *Phymatotrichum omnivorum* (Shear) Dugg, than did very young seedlings. Shaw (19) observed that apple shoots decreased in susceptibility to fire blight with age. Anderson (1) found no differences in susceptibility to yellows (*Fusarium oxysporum* f. *conglutinans* (Wr.) S. & H.) in cabbage plants of different ages. In certain cases, greater disease development has been observed on the more vigorously growing host plants, as pointed out by Piemisel (14), Kyle (12), Raines (16, 17), and Shaw (19). Pritchard and Porte (15) stated that favorable conditions for growth of tomato plants increased their susceptibility to leaf spot (*Septoria lycopersici* Speg.). In direct contrast to the foregoing, Dickson, Eckerson, and Link (7) found that low vegetative vigor predisposed corn and wheat seedlings to infection by *Gibberella saubinetii*.

The purpose of this investigation was to determine in what way and to what extent certain factors in the environment may bring about predisposition to fusarium wilt in tomato plants. By studying the effects of variations in the environment given the host plant only before infection, an attempt was made to determine the effect of environment on disease development through its action upon the host alone and to eliminate as much as possible the effects of environment on the disease complex. This was accomplished by varying a given factor in the environment in which healthy young tomato plants were grown for a period before inoculation, and studying the development of disease after infection when the plants were growing under uniform conditions for wilt development.

The term "predisposition," as employed in this paper, refers to any modification of the host plant brought about by environmental or nutritional factors previous to infection which influences the expression of the disease symptoms after infection. An attempt was made in this investigation to distinguish such effects from those of environment and nutrition after infection and from those of inherent degrees of resistance or susceptibility characteristic of certain strains of the host plant.

#### MATERIALS AND METHODS

In all experiments, three types of hosts were used. Bonny Best (*Lycopersicon esculentum* Mill.) represented the class very susceptible

to wilt; Marglobe (*L. esculentum*), which has been used commercially with a fair degree of success in many wilt-infested areas, represented the class of varieties which have an intermediate degree of resistance; a strain of Red Currant tomato (*L. pimpinellifolium* Mill.) represented those strains or varieties highly resistant to wilt, in which resistance is inherited as a qualitative character and controlled by a single gene (3).

These hosts were sown in soil free from the wilt organism or in washed sterilized fine white silica sand, and were transplanted approximately 3 weeks later when the first true leaves were forming. The plants were grown for a period of from 30 to 50 days under environmental conditions which were near the optimum for vigorous growth except for the single predisposing factor being varied. Predisposing conditions will be described for each series of experiments under Experimental Results. All other conditions not under test were kept as uniform as possible. At the end of the predisposing period, all plants were inoculated with a virulent strain of the organism according to the method outlined by Walker and Foster (24). After inoculation, the plants were all given the same environmental conditions, which were maintained as near as possible to the optimum for the development of wilt, unless otherwise indicated. Disease indices were calculated (24) for each type of host grown under each predisposing condition. At least 20 plants of each host type were given each variation in environment in each experiment, and 2 or more experiments were conducted for each predisposing condition under test.

## EXPERIMENTAL RESULTS

### PREDISPOSITION BY SOIL TEMPERATURE

Ten plants of each host type were grown in clean compost soil in each of 8 crocks in a Wisconsin soil temperature tank. Soil in the crocks was insulated from the air by a 1-inch layer of ground cork and the moisture content was maintained at the optimum by adding enough water daily to bring the crocks up to original weight plus estimated plant weight. Tanks were adjusted to maintain soil temperatures of 12°, 20°, 28°, and 36° C., while a common air temperature of about 24° was maintained. Plants were grown at these soil temperatures for 50 days and were then inoculated. The tomatoes grown at soil temperatures of 20° and 28° were large, vigorous, and succulent; those grown at either 12° or 36° were small, less succulent, and of low vigor (fig. 1, A).

After inoculation, the crocks were rearranged so that each temperature tank contained 2 crocks of plants predisposed at each soil temperature. Thus, for example, the 20° tank had 20 plants predisposed at 12°, 20 predisposed at 20°, 20 at 28°, and 20 at 36°. This set-up was duplicated for each host type, and disease development indices were recorded for each type of host predisposed at each soil temperature and under each postinoculation soil temperature condition. The disease development curves for Bonny Best and Marglobe plants grown at each predisposing soil temperature and held at 20° soil temperature after inoculation are shown in figure 2. Plants of both



FIGURE 1.—*A*, Representative 'Bonny Best' plants grown for 50 days before inoculation at the following soil temperatures: *a*, 12° C.; *b*, 20°; *c*, 28°; and *d*, 36°. *B*, Plants grown at the same temperatures and held at 20° soil temperature for 28 days after inoculation, with other environmental conditions near optimum.

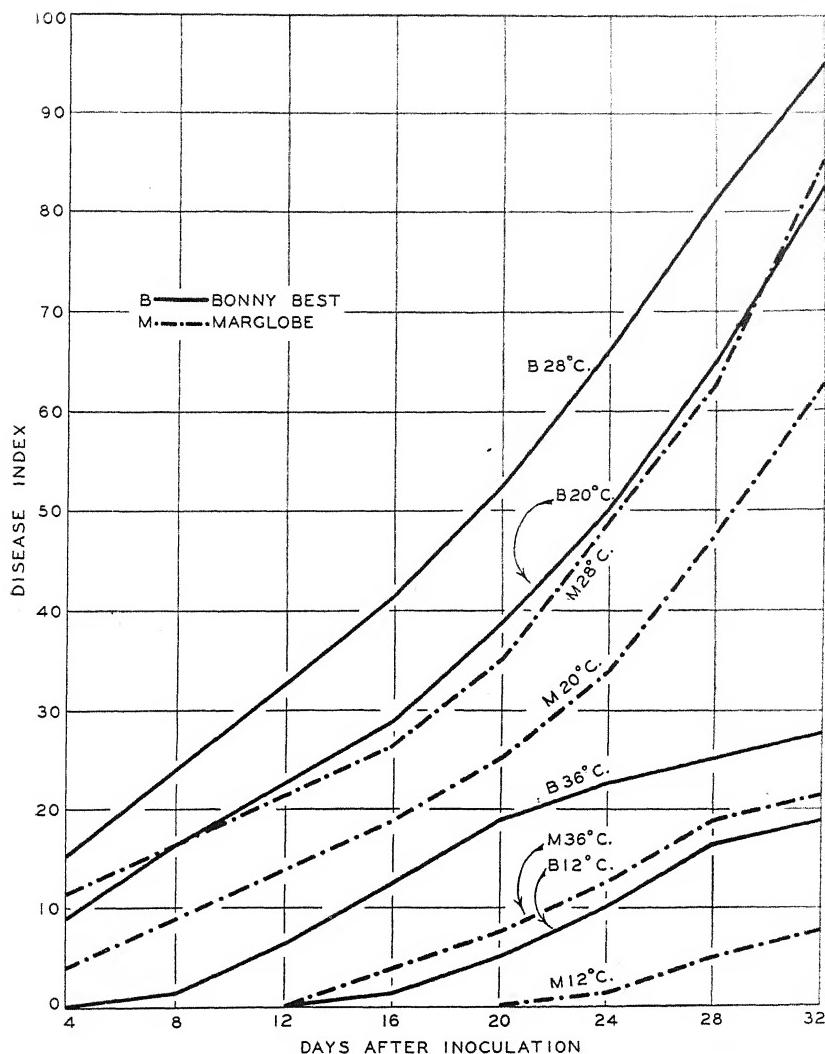


FIGURE 2.—Progression of wilt development in Bonny Best and Marglobe plants predisposed for 50 days at various soil temperatures (indicated for each curve), and held after inoculation at 20° soil temperature with all other environmental conditions near optimum.

Bonny Best and Marglobe predisposed at 28° wilted most severely; those predisposed at 20° wilted less; those predisposed at 36° still less; and those predisposed at 12° wilted least. Bonny Best predisposed at 20° and Marglobe at 28° showed approximately the same degree of susceptibility. Red Currant retained its immunity under all conditions tested. The same relative susceptibility was manifested between varieties and between predisposing temperatures at all temperatures maintained after inoculation. Thus, Bonny Best plants

always developed disease to a greater extent than Marglobe plants given identical treatments. Under postinoculation temperatures of 12°, 20°, 28°, and 36°, respectively, those plants of either variety predisposed at 28° were the most susceptible, with a decrease in susceptibility in plants predisposed at 20°, 36°, and 12° in the order mentioned. However, total disease development was greater for all entities in the 28° postinoculation temperature, less at 36°, and still less at 12°. Wilt development in Bonny Best plants predisposed at the 4 different soil temperatures is illustrated in figure 1, B. A second experiment yielded similar results.

#### PREDISPOSITION BY AIR AND SOIL TEMPERATURE

A second series of experiments was conducted in a manner similar to the soil temperature experiments except that plants were predisposed in pots in greenhouses with constant air temperatures at 16°, 20°, 24°, and 28° C., where tops and roots were exposed to approximately the same temperatures, the soil temperature being slightly below that of the air temperature as a result of the cooling effect of



FIGURE 3.—A, Bonny Best plants grown for 50 days before inoculation at the following soil and air temperatures: a, 16° C.; b, 20°; c, 24°; and d, 28°. B, Plants grown at the same temperatures and held at 24° soil and air temperature for 24 days after inoculation; all other conditions were near optimum.

evaporation. The largest, most vigorous, and most succulent plants were produced at 28° with a decrease in size, vigor, and succulence with each successive drop in predisposing temperature (fig. 3, A). After inoculation, plants predisposed at all of these temperatures were placed in each of the houses and wilt development was recorded for each unit. Disease index curves from a typical experiment with Bonny Best and Marglobe, predisposed at the four different temperatures and held at 28° after inoculation, are shown in figure 4. Plants

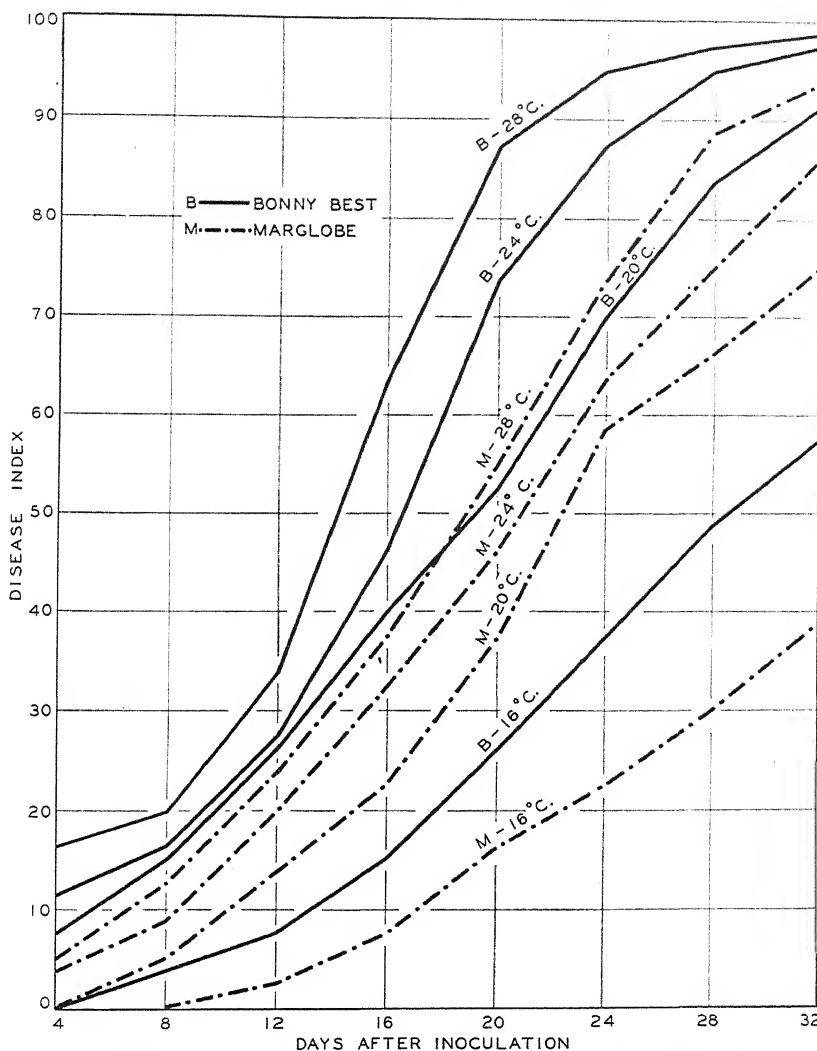


FIGURE 4.—Progression of wilt development in Bonny Best and Marglobe plants predisposed for 50 days at various soil and air temperatures (indicated for each curve), and held after inoculation at 28° soil and air temperature; all other conditions were near optimum.

predisposed at the same four air temperatures and given postinoculation temperature conditions of 24°, 20°, and 16° showed the same relative susceptibility. Total disease development during the term of the experiment, however, decreased as the postinoculation temperature was lowered. Tomatoes were most effectively predisposed to wilt at 28°, less so at 24° and 20°, and least at 16°. The disease reactions of Bonny Best predisposed at 20° and Marglobe at 28° were similar. Red Currant failed to develop wilt under any combination of conditions. Figure 3, B, shows wilt development in Bonny Best plants

predisposed at the four temperatures and grown at a 24° postinoculation soil and air temperature.

Through a combination of the methods used in the experiments described above, predisposing effects of soil temperature and air temperature were studied individually. The results were similar to those obtained in the other experiments, i. e., temperatures most favorable to tomato plant growth were most favorable to wilt. However, the effect of air temperature alone was somewhat less pronounced than that of soil temperature alone in predisposing the plants to wilt.

#### PREDISPOSITION BY SOIL MOISTURE

Plants were grown before inoculation in clean compost soil in glazed crocks. The soil was adjusted to three levels of moisture comparable to those used by Clayton (4), i. e., dry (approximately 15 percent of wet weight), optimum (approximately 30 percent), and very wet (approximately 37 percent). These moisture levels were maintained by

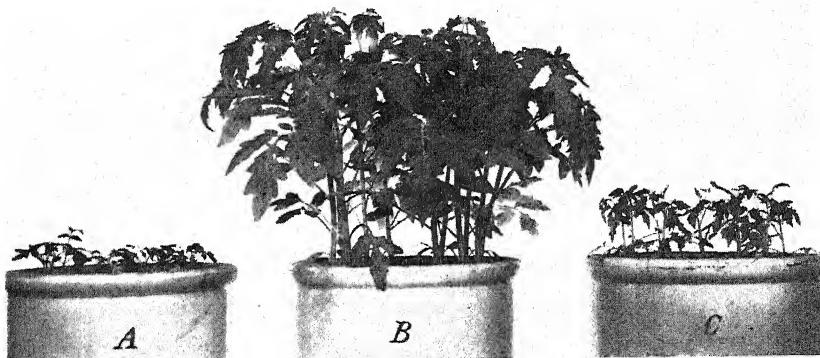


FIGURE 5.—Bonny Best plants grown for 30 days before inoculation in soil of various moisture levels: A, dry; B, optimum; C, very wet; other environmental conditions were near the optimum.

adding enough distilled water to the crocks each morning to bring them up to the desired weight and adding measured amounts of water each afternoon. The dry soil did not contain enough moisture to prevent flagging of the plants entirely on bright days. Plants grown in dry soil were of low vigor and were least succulent; those grown in wet soil were also low in vigor, etiolated, and spindly. Soil of optimum moisture content produced normal, vigorous plants (fig. 5).

After being grown under the different soil moisture conditions for 30 days, plants were inoculated, transplanted into new soil in pots, and given optimum soil moisture as well as optimum soil temperature. Wilt development was recorded as before. Disease development curves from a typical experiment are shown in figure 6 for Bonny Best and Marglobe predisposed at the three soil moisture levels. With both the susceptible and the intermediate-resistant hosts, dry soil predisposed the plants to wilt, whereas very wet soil decreased potential susceptibility. No disease developed in Red Currant tomato.

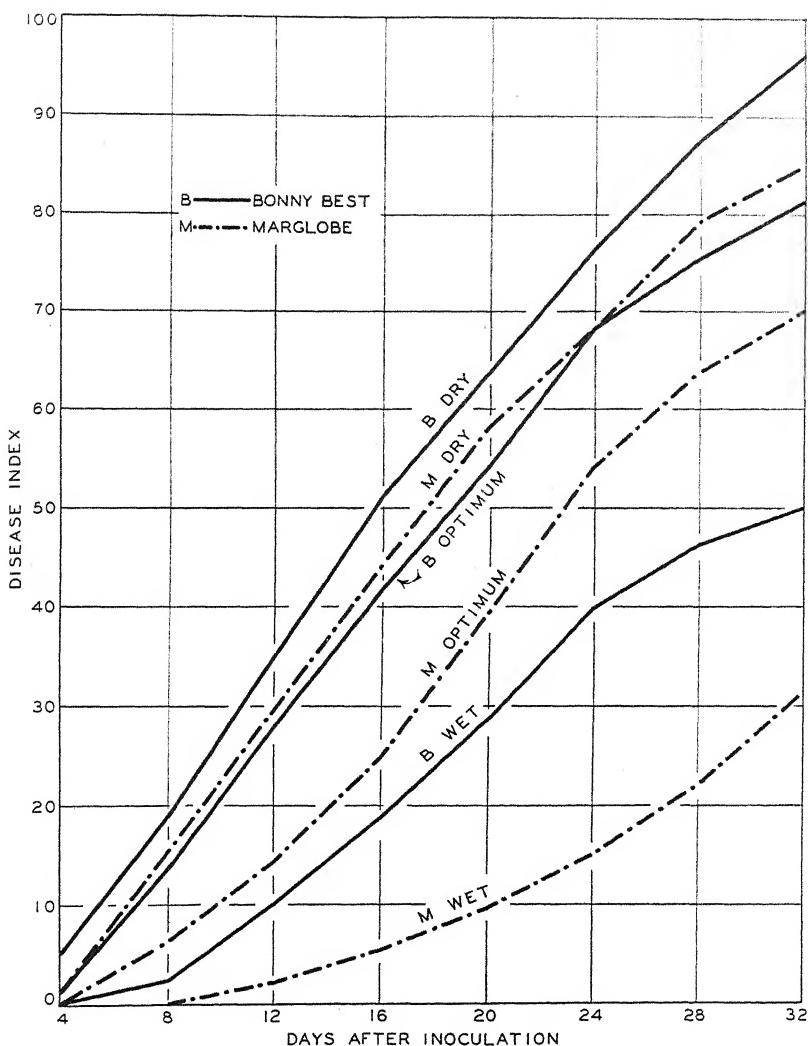


FIGURE 6.—Progression of wilt development in Bonny Best and Marglobe plants predisposed for 30 days in soil of various moisture levels (indicated for each curve) and held after inoculation in soil of optimum moisture content; other conditions were also optimum for wilt.

## PREDISPOSITION BY LENGTH OF DAY

Eighty plants of each host type were grown for 30 days before inoculation in either a long or a short day. The plants grown in a long day received sunlight, supplemented by fluorescent light morning and evening, for a total light period of 18 hours each day. Plants grown in a short day were covered by a ventilated dark cage from 4 p. m. until 10 a. m., central war time, each day. Long-day plants were vigorous, stocky, and dark green in color; short-day plants were lower in vigor and somewhat etiolated (fig. 7).

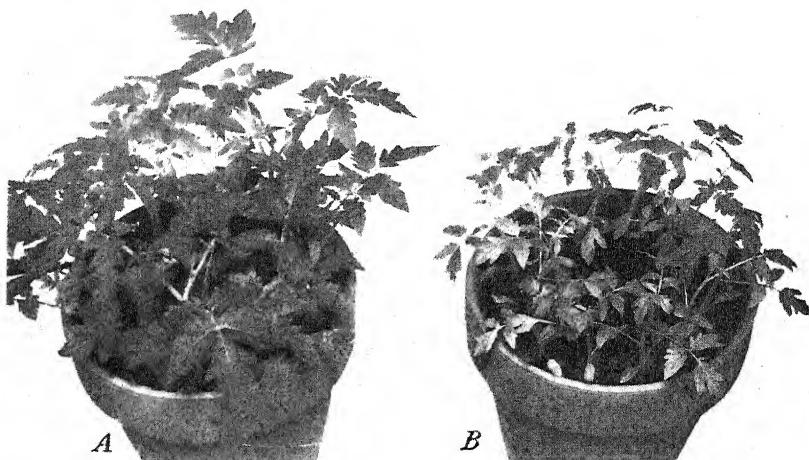


FIGURE 7.—Bonny Best plants grown for 30 days before inoculation in two different photoperiods: *A*, 18-hour day; *B*, 6-hour day; other environmental conditions were near the optimum.

After inoculation, both sets of plants received a 12-hour light period. Disease development was recorded as before; the data from a typical experiment are presented graphically in figure 8 for Bonny Best and Marglobe predisposed in the different day lengths. In both varieties effective predisposition to wilt was brought about by the short-day condition. No evidence of wilt was observed in either set of Red Currant plants.

## PREDISPOSITION BY LIGHT INTENSITY

Plants were grown under 2 different conditions of light intensity for 30 days before inoculation. A low light intensity of approximately 90 foot-candles (range 60-110) was given 1 set of 80 plants of each host type by keeping them covered with a muslin cage. A comparable set received light of high intensity (approximately 800 foot candles, range 600-900) furnished by sunlight supplemented with artificial light from 200-watt Mazda bulbs. Plants grown in the light of greater intensity were normal and vigorous; those grown under reduced light were of low vigor and slightly etiolated (fig. 9).

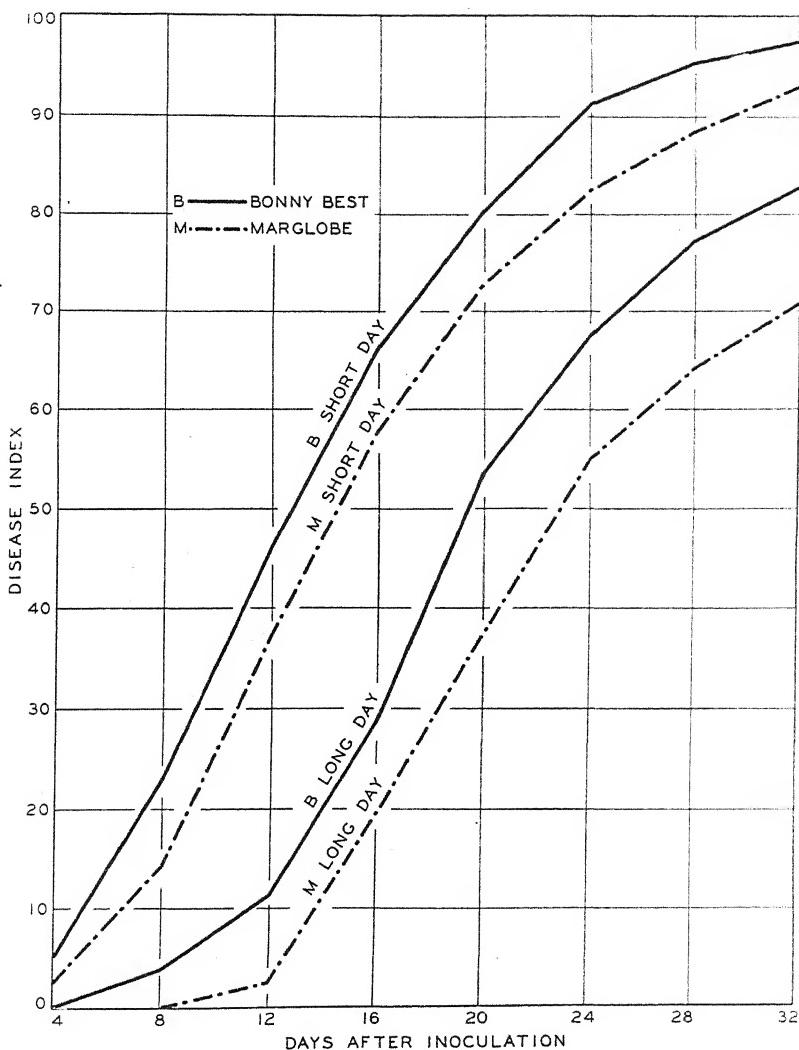


FIGURE 8.—Progression of wilt development in Bonny Best and Marglobe plants predisposed for 30 days in two different photoperiods (indicated for each curve), and given an intermediate-day length after inoculation; all other environmental conditions were maintained near the optimum.

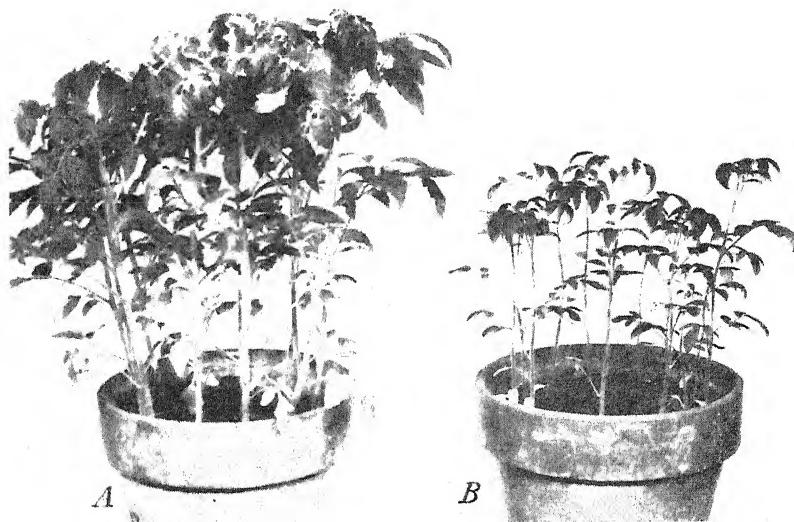


FIGURE 9.—Bonny Best plants grown for 30 days before inoculation under two different light intensity conditions: A, 800 foot-candles; B, 90 foot-candles; all other environmental conditions were near optimum.

After inoculation, both sets of plants received only sunlight (approximately 550 foot-candles, range 260–670). Wilt-development data from one of two experiments conducted are presented in figure 10 for Bonny Best and Marglobe grown under the two light intensities. Plants receiving less light were predisposed to wilt to a greater degree than those conditioned under light of high intensity. As in other experiments, Red Currant plants remained healthy.

#### PREDISPOSITION BY NUTRIENT BALANCE

Plants of the three host types were grown in sand and transplanted into drip-system pots where they were given one of seven different nutrient solutions as outlined by Walker and Foster (24) for 30 days before inoculation. The solutions used were balanced, high N, low N, high K and low K described earlier (24) and a high P solution containing three times as much phosphorus as the balanced solution, and a low P nutrient containing one-tenth the amount of phosphorus in the balanced solution. In all of the unbalanced solutions, none of the varied ions was ever lacking completely, and all other necessary ions were kept at the level found in the balanced solution. In all solutions the pH was adjusted to 6.5–7.0, and all were regulated to the same osmotic concentration by the addition of NaCl. In no case did the total Na<sup>+</sup> or Cl<sup>-</sup> ions approach an amount not readily tolerated by tomato plants.

Plants receiving the unbalanced solutions varied in size, but not in vigor or succulence, from those grown in the balanced solution. Plants grown in the high N, low N, high K and low K solutions were

somewhat smaller than the controls, while the plants grown before inoculation in the high or low P solutions were of the same approximate size as the controls. Excess or deficiency symptoms did not

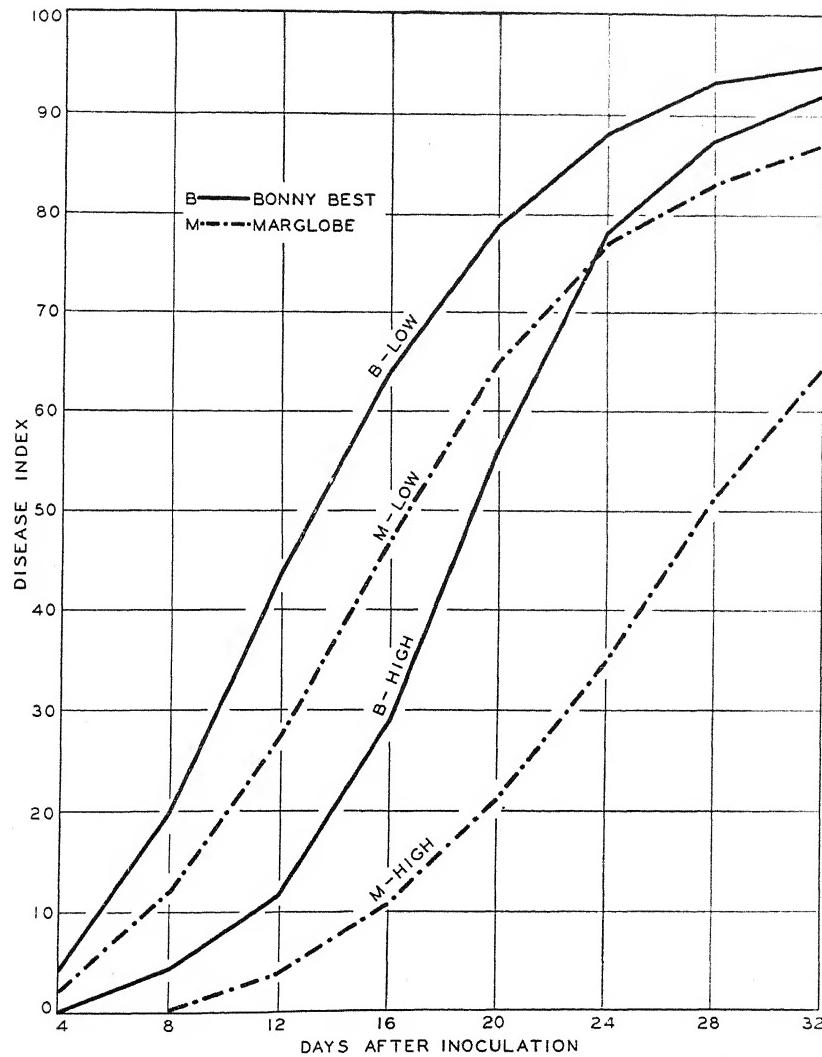


FIGURE 10.—Progression of wilt development in Bonny Best and Marglobe plants predisposed for 30 days under two different light intensities (indicated for each curve), and given an intermediate light intensity after inoculation all other environmental conditions were near optimum.

develop in any of the plants. Following inoculation, the solution supply lines were cleaned thoroughly and the balanced solution was given to all plants. Disease development was recorded in the usual

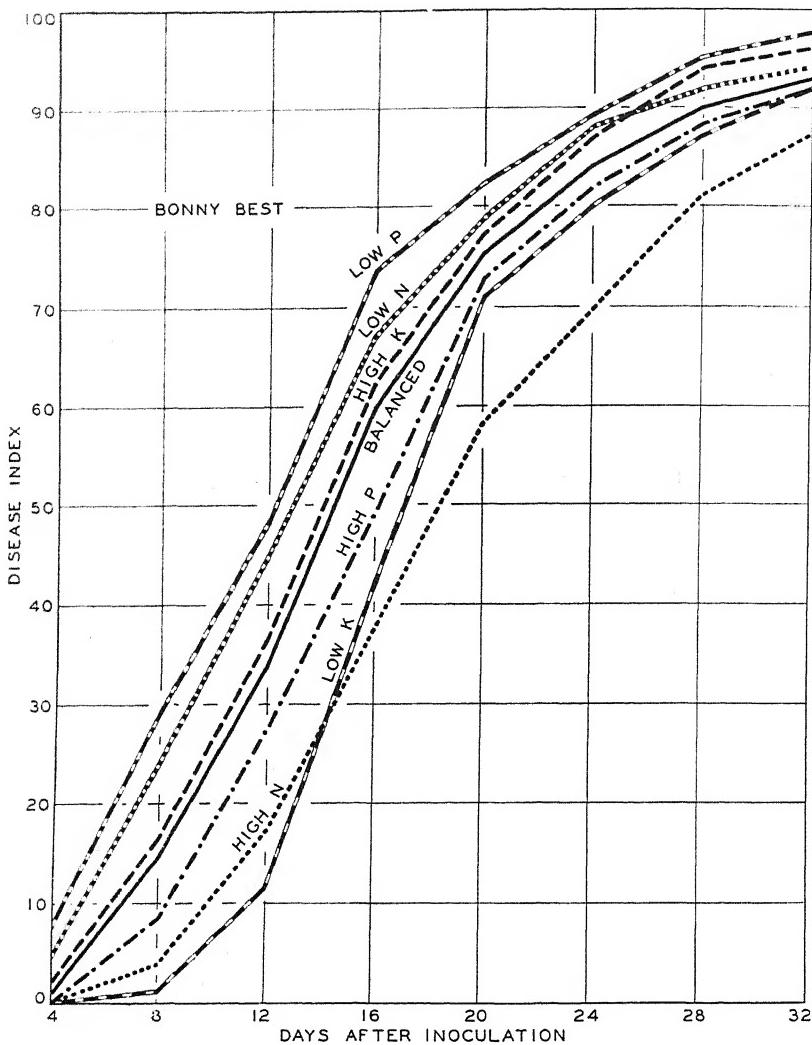


FIGURE 11.—Progression of wilt development in Bonny Best plants predisposed for 30 days before inoculation in various unbalanced nutrient solutions (indicated for each curve) and all given a balanced solution after inoculation; all other conditions were near optimum.

manner; data from a typical experiment are presented graphically for Bonny Best in figure 11 and for Marglobe in figure 12.

Predisposition most favorable to subsequent wilt development in both Bonny Best and Marglobe was brought about by low N, high K, and low P nutrition as compared with the balanced solution or with high N, low K, or high P, respectively. Decrease in potential susceptibility to wilt was found to have been conditioned in the plants by the solutions in the following order: Low P (most susceptible),

low N, high K, balanced solution, high P, low K, high N (least susceptible). Red Currant remained immune in all solutions.

#### PREDISPOSITION BY HYDROGEN-ION CONCENTRATION OF THE NUTRIENT

Because of the difficulty often experienced in adjusting and maintaining soil at a given pH level, these experiments were conducted in the drip-system along with the nutrition experiments. Balanced nutrient solutions were used, being adjusted in one case to pH 8.5 and

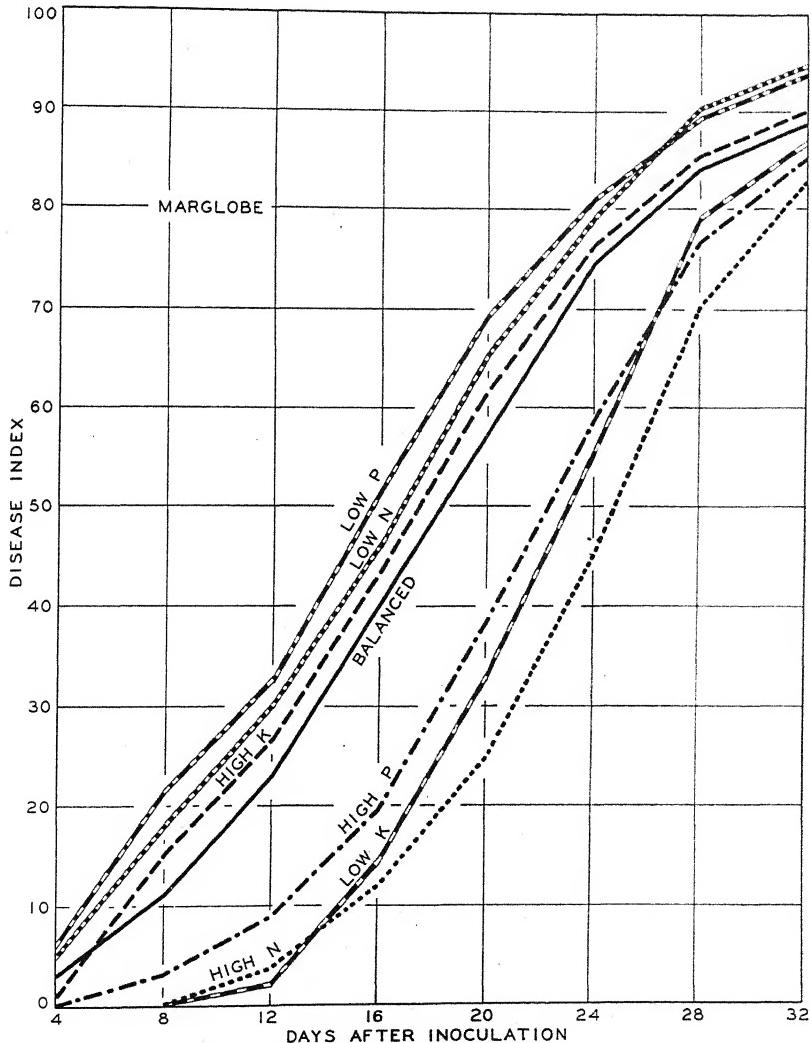


FIGURE 12.—Progression of wilt development in Marglobe plants predisposed for 30 days before inoculation in various unbalanced nutrient solutions (indicated for each curve) and all given a balanced solution after inoculation; all other conditions were near optimum.

in the other to pH 4.5. Plants grown in the balanced solution at pH 6.5-7.0 were used for comparison. Plants were grown for 30 days in these solutions before inoculation. Those grown in the high or low pH nutrients were slightly smaller than the plants grown in the balanced solution, but all were of normal color and succulence. Severe stunting and reduced vigor as described by Sherwood (21) for tomato plants grown in soil of high pH were not evident in the plants in this experiment. After inoculation, all plants received the balanced solution at pH 6.5-7.0. Wilt indices for Bonny Best and Marglobe are shown in graphic form in figure 13.

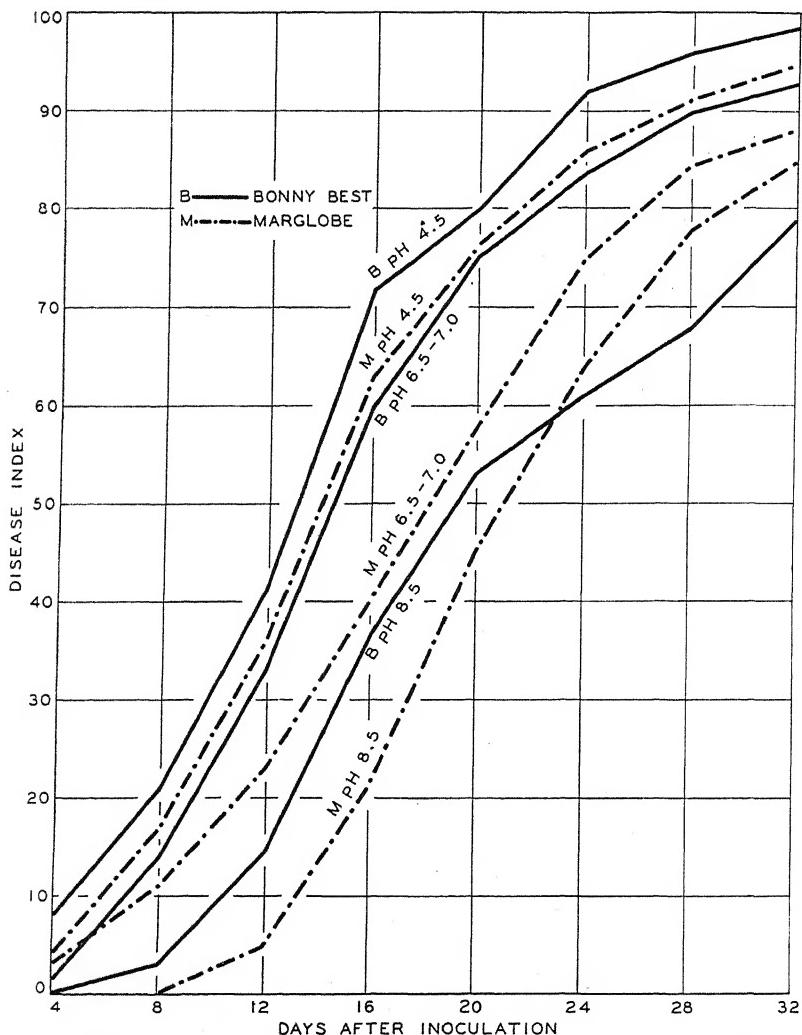


FIGURE 13.—Progression of wilt development in Bonny Best and Marglobe plants predisposed for 30 days before inoculation in balanced nutrient solutions of various hydrogen-ion concentrations (indicated for each curve), and given a solution of pH 6.5-7.0 after inoculation; all other conditions were near optimum

In both Bonny Best and Marglobe, plants grown in the low pH solution were more susceptible, while those grown in the high pH solution were less susceptible than those grown in the solution at pH 6.5-7.0. Neither predisposing condition had any effect on altering the complete resistance of Red Currant.

#### RELATION OF AGE OF HOST TO PREDISPOSITION

In order to study further the relation between size and vigor of the host and its predisposition to fusarium wilt, plants of different ages were inoculated, and disease development was recorded. Seeds of all 3 host types were sown at 10-day intervals and seedlings were transplanted into flats of clean compost soil 10 days after emergence and held under optimum conditions until 50-, 40-, 30-, 20-, and 10-day old plants were at hand. These plants varied in size and represented the range in size of plants obtained in all previous experiments. The plants, however, were all of normal color and succulence and were neither stocky nor etiolated. After inoculation, the plants were returned to the flats of soil and wilt development was recorded as before.

In neither Bonny Best nor Marglobe did age of plants have any significant or consistent effect upon wilt development. All plants of both varieties appeared to succumb to the disease at about the rate typical of the variety, although evaluation of disease development was difficult for the small plants. Age at the time of inoculation had no effect upon Red Currant since all plants of this strain remained healthy.

#### DISCUSSION

The complexities encountered in studying the effects of environmental factors upon disease development emphasize the need for separating, if possible, the effects of such factors upon the host from those upon the host-parasite complex. An attempt to do this was made in these studies by subjecting healthy tomato plants to variations of one factor in the environment at a time, introducing the parasite, and then studying the reaction of these plants in an environment held, without variation, near the optimum for disease development. Differences in disease reaction of these plants could then be attributed to the effect of environment on the plants alone, since the parasite and the host after inoculation received the same set of environmental factors at all times. By this method, most of the major environmental factors were found to influence disease development through their action on the tomato plant as indicated earlier by Foster (10). Most of the factors which increased disease development in infected plants also increased the potential susceptibility of healthy plants, but for several of the factors, the reverse reaction was apparent. When soil temperatures alone, air temperatures alone, or soil and air temperatures combined, were varied, young tomato plants grown at levels near the optimum for growth of the host and for disease development were most favorably predisposed to wilt. On the other hand, the plants low in vigor grown in dry soil before infection were more favorably predisposed than the plants of high vigor grown in soil of optimum moisture content, while the plants of low vigor grown in very wet soil were the least favorably predisposed to the disease. The increase in susceptibility brought about in plants

grown in dry soil prior to infection is in contrast to the effects of soil moisture upon disease development after infection as reported by Clayton (4), who found wilt development to be retarded by both high and low soil moistures.

In the study of predisposition by day length and by light intensity, plants that received least light were found to be the most susceptible, a relation which is similar to that observed with infected plants growing in the greenhouse during winter months as contrasted with those growing during spring or summer months. Regarding these two factors, potential susceptibility of the hosts appeared to be correlated with low vigor. Under conditions of low light intensity or short light periods, the carbon-nitrogen ratio in the plants would be altered in favor of increased nitrogen. Therefore, the increase in susceptibility might have been brought about by the relatively higher nitrogen ratio in the plants. This indication regarding the relationship of the nitrogen ratio to potential susceptibility was not supported, however, by the results of experiments on predisposition by nutrient balance.

Walker and Foster (24) showed that infected tomato plants receiving unbalanced nutrients high in nitrogen or low in potassium developed wilt most severely as compared with infected plants grown in a balanced nutrient or in solutions low in nitrogen or high in potassium. In direct contrast, it has been shown in these studies that plants grown before inoculation in a solution low in nitrogen became more susceptible to wilt and those receiving nutrient high in nitrogen became less susceptible. The fact that high and low potassium solutions conditioned a comparable reversal in predisposing tomato plants suggests that these two elements are interdependent in some manner and may affect the potential susceptibility of the tomato to the wilt pathogen through their action upon a common factor. Shear and Wingard (20) pointed out that, according to several workers, deficiency of phosphorus resulted in an accumulation of nitrogen in tomato plants. Results of experiments conducted in this study are not in accord with this concept since the solution low in phosphorus brought about the same type of predisposition as did the low nitrogen nutrient, and also the high phosphorus and high nitrogen solutions had comparable effects on the potential susceptibility of the host. The effects of nitrogen and phosphorus were found to be similar in relation to disease development of cabbage yellows (25) but different in cabbage clubroot (26). In the studies on predisposing tomato plants by unbalanced nutrition, no correlation between host size or vigor and susceptibility was apparent.

In sand culture, tomatoes grown in a solution of high pH were less susceptible to wilt; those grown in a low pH nutrient were more susceptible. While the relationship of susceptibility to pH may be different in plants grown in soil, these findings are in accord with those of Edgerton and Moreland (9), who reported a decreased wilt development in tomatoes grown on limed soil. Also Sherwood (21) presented evidence that wilt was most severe in tomato plants grown on acid soils. Again, the somewhat smaller and less vigorous plants grown in solutions of either high or low pH were more or less susceptible depending upon the treatment they had received before inoculation.

That the size and vigor of tomato plants are not consistently correlated with susceptibility to wilt is shown by the preceding experiments. This was substantiated still further in the experiments on age of plants in relation to predisposition. Plants varying in age and thus in size all showed approximately the same potential susceptibility, which leads to the conclusion that predisposition to wilt may be induced in tomato plants by a number of major factors in the environment and that variation in those factors may or may not affect the size and vegetative vigor of the plants.

Susceptible Bonny Best and intermediate resistant Marglobe were predisposed in very nearly the same manner by variations in the environment. Under a certain set of environmental conditions, Marglobe may be made to appear as susceptible as Bonny Best grown under another set of conditions. Similarly, by controlling the environment in which it is grown, Bonny Best may be reduced in potential susceptibility to the level normally shown by Marglobe. From this it is apparent that the type of resistance carried by Marglobe is variable, and a similarity to type B resistance of cabbage to yellows (1, 25) is suggested. Since Red Currant plants showed no evidence of the disease under any of the conditions tested, it is to be concluded that these plants carry a type of resistance closely approaching true immunity and that this high degree of resistance may be attributed to a character or characters little influenced in their expression by the environment.

In tomato plants inherently susceptible or partially resistant to *Fusarium oxysporum* f. *lycopersici*, a wide range of disease reaction has been obtained by varying the environment acting upon the host previous to inoculation and not upon the pathogen or the host-pathogen complex. This leads to the conclusion that the relation of environment to disease development in many cases may be attributed, in part at least, to the effect of environment in the host alone, while in other cases, factors in the environment apparently assert their over-all effects largely upon the host-pathogen complex, overcoming changes in potential susceptibility or resistance conditioned in the host prior to infection. The nature of predisposition occasioned by temperature, light, and high water content, and pH of the substrate are examples of the former possibility; low soil water and unbalanced nutrition exemplify the latter.

It is recognized that, in the greenhouse experiments conducted in this study, certain extremes in conditions were used to facilitate recognition of the effects of environmental factors. Methods of growing and inoculating plants were often such as are infrequently encountered in the field. While direct comparisons cannot be made in all cases with field-grown plants, tendencies or trends in altered potential susceptibility occasioned by certain environmental factors have been clearly demonstrated and the phenomenon of predisposition has been shown to be of major importance in the consideration of environment in relation to wilt development.

#### SUMMARY

By subjecting healthy young tomato plants to variations in the environment only before inoculation and then maintaining a constant

and optimum set of conditions for wilt development after inoculation, the effect of environmental factors on disease development through action upon the host alone was studied. Tomatoes were found to be favorably predisposed to wilt (*Fusarium oxysporum* f. *lycopersici* (Sacc.) S. & H.) by the following entities of the environment: (1) Soil or air temperature near the optimum for plant growth, (2) low soil moisture, (3) short day length, (4) low light intensity, (5) nutrient low in nitrogen, low in phosphorus, or high in potassium, and (6) nutrient low in pH. Potential susceptibility of the plants was found to be decreased and consequently their resistance increased by soil or air temperatures above or below the optimum for plant growth, by very wet soil, by long daylight periods, and by high light intensity. Decreased susceptibility was also conditioned in plants grown in solutions high in phosphorus, low in potassium, or high in nitrogen and also in solutions with a high pH.

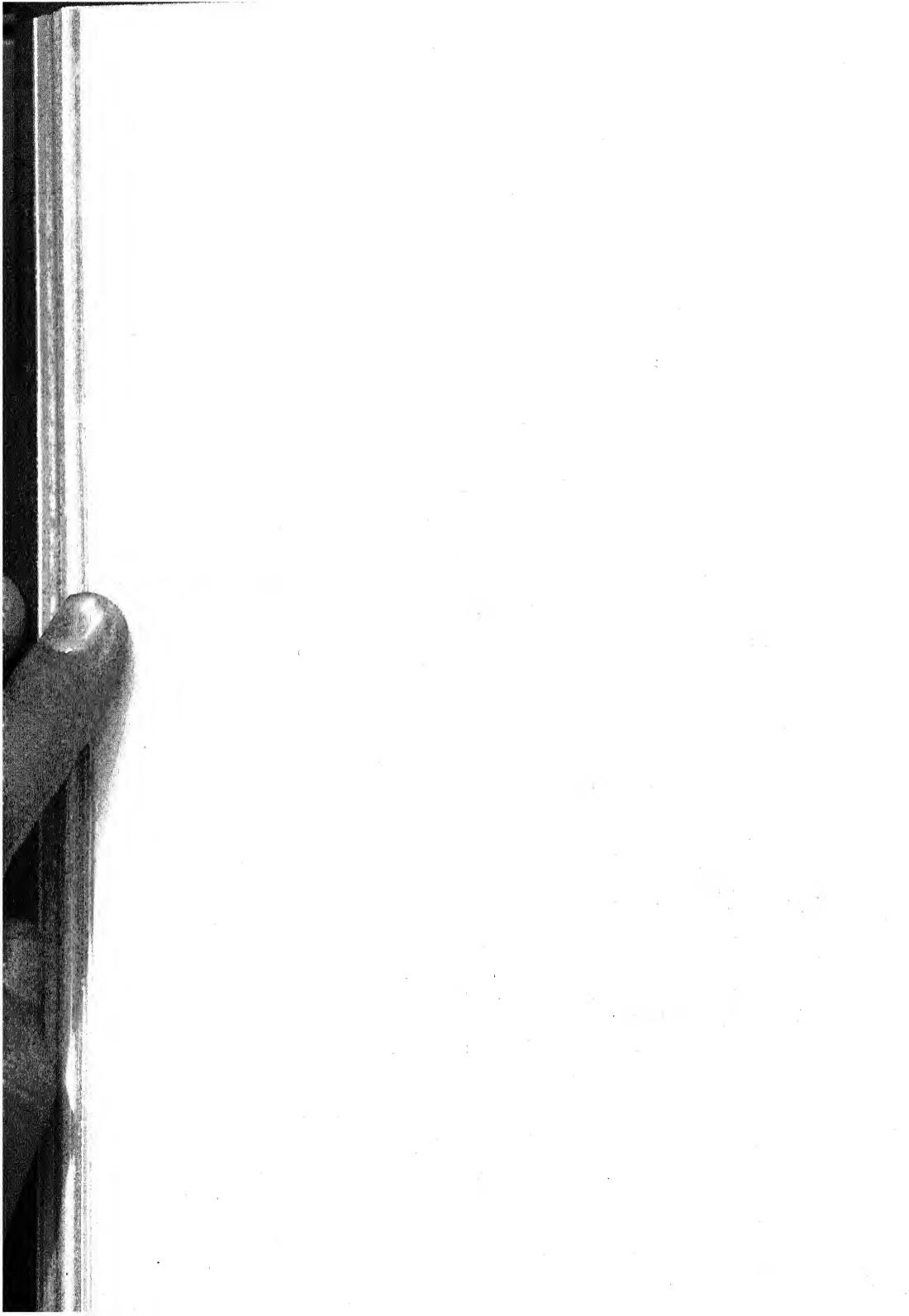
Neither size nor vigor of the host as determined by particular variations in environmental conditions or by age of the plants at the time of inoculation showed any consistent correlation with subsequent wilt development.

Susceptible Bonny Best and intermediate-resistant Marglobe were both predisposed to wilt by the same environmental factors and apparently in the same manner. Resistant plants were made to appear susceptible, or normally susceptible hosts were conditioned so as to appear resistant, by proper control over the environment in which they were grown prior to infection. It was not possible to alter the high degree of resistance found in the strain of Red Currant tomato used.

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# EFFECT OF INDOLE-3-BUTYRIC ACID ON TRANSPLANTED PECAN TREES<sup>1</sup>

By C. L. SMITH

*Physiologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture*

## INTRODUCTION

Recently indole-3-butyric acid was applied to roots of nursery-grown trees of pecan (*Carya illinoensis* (Wang.) K. Koch) at the time of transplanting to stimulate root formation. During the experimentation<sup>2</sup> it was found that when 8 mg. of the acid per tree was applied to the taproots the trees averaged 39 percent more new shoot and leaf growth than did similar trees treated with 16 mg. of the acid, but it was not clear whether the larger amounts of indole-3-butyric acid inhibited new top growth directly or stimulated new root formation and growth and thus indirectly inhibited top growth by utilizing greater amounts of the stored nutrient materials. Marth<sup>3</sup> found that certain growth-regulating chemicals applied to the whole plant effectively inhibited growth of buds on rose bushes during 40 to 60 days in common storage and that the starch content of the treated bushes was conserved during storage; as a result the treated bushes made more root and shoot growth when transplanted to the field than did similar untreated ones.

In order to determine the effects of indole-3-butyric acid on the composition of the tissues of transplanted pecan trees, experiments were conducted during the 1944 growing season at Brownwood, Tex.

## MATERIALS AND METHODS

Seedling pecan trees 4 to 6 years old were selected and divided into seven uniform lots. Each lot contained six 6-year-old trees and eight 4-year-old ones; the trees were selected for uniformity of size and for type of root system and top. They were dug on April 5 and 6 and carefully heeled in. On April 7 the taproots of three lots were treated with indole-3-butyric acid, and they and three similar untreated lots were then transplanted to the field. The treatments consisted of boring small holes at four different places in the taproot of each tree and

<sup>1</sup> Received for publication August 17, 1945.

<sup>2</sup> SMITH, C. L., and ROMBERG, L. D. THE USE OF INDOLEBUTYRIC ACID AS AN AID IN TRANSPLANTING PECAN TREES. Tex. Pecan Growers Assoc. Proc. 21: 17-22. 1941.

<sup>3</sup> MARTH, P. C. EFFECTS OF GROWTH-REGULATING SUBSTANCES ON SHOOT DEVELOPMENT OF ROSES DURING COMMON STORAGE. Bot. Gaz. 104: 26-49, illus. 1942.

inserting in each one-half of a round toothpick containing approximately 2 mg. of indole-3-butyric acid. Thus, 8 mg. of the chemical was inserted in the taproot of each treated tree. The toothpicks were prepared by soaking in an alcoholic solution of the acid and then allowing the alcohol to evaporate.<sup>4</sup>

One lot was sampled for analysis at the beginning of the experiment, on April 7. One lot each of treated and untreated trees was dug and sampled for analysis on June 28, on September 5, and on October 12, in order to follow the progressive changes in composition during the growing season. The new roots, shoots, and leaves were removed at the time of digging, and the dry weights were determined. Samples of trunks, including wood and bark, were taken by sawing through the trunk of each tree in the lot, collecting the sawdust, and composting it for sampling. Those of taproot wood were taken similarly after the bark had been stripped from a 12-inch section at the middle of each. The taproot bark from all the sections was chopped fine with shears and after thorough mixing was sampled for analysis.

Analyses were made of dry matter, reducing and nonreducing sugars, starch, hemicellulose,<sup>5</sup> and organic nitrogen. The methods of preserving the samples and of analyses were the same as those used by Thor and Smith<sup>6</sup> except as stated. Organic nitrogen was determined by the official Kjedahl-Gunning-Arnold method,<sup>7</sup> and the cuprous oxide in the analyses of sugars, starch, and hemicellulose was determined by direct weighing. Dry matter was calculated as a percentage of fresh weight, and all other elements were calculated as percentages of dry matter.

## EFFECT OF TREATMENT ON COMPOSITION

### DRY MATTER

The percentages of dry matter in the trunks of treated and untreated trees were approximately the same except on June 28 (fig. 1, A). On that date the percentage had increased considerably in untreated trees, while in the treated ones there was a corresponding decrease. Although the percentages in the wood of the taproots decreased generally throughout the season, the decrease was greater in treated trees than in untreated ones. The percentages were always higher in the bark of taproots of untreated trees than in that of treated ones, although the fluctuations were in different directions on June 28 and September 5.

### REDUCING SUGARS

The percentages of reducing sugars were low and fluctuated similarly in the trunks of treated and untreated trees (fig. 1, B). Those in the taproot wood increased in both cases, but on all sampling dates the percentages for the treated trees were about three times those for

<sup>4</sup> See footnote 2, p. 187.

<sup>5</sup> Hemicellulose as used here includes the carbohydrate bodies hydrolyzed by dilute mineral acids but not by diastase.

<sup>6</sup> THOR, C. J. B., and SMITH, C. L. A PHYSIOLOGICAL STUDY OF SEASONAL CHANGES IN THE COMPOSITION OF THE PECAN DURING FRUIT DEVELOPMENT. *Jour. Agr. Res.* 50: 97-121, illus. 1935.

<sup>7</sup> ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS . . . Ed. 5, 757 pp., illus. Washington, D. C. 1940.

the untreated ones. Although the percentages were higher in the taproot bark of untreated trees than in that of the treated ones, the fluctuations were similar.

#### NONREDUCING SUGARS

The curves for the nonreducing sugars in the trunks and taproot bark of treated trees differed little from the corresponding curves for untreated ones (fig. 1, C). The percentages in the taproot wood of the

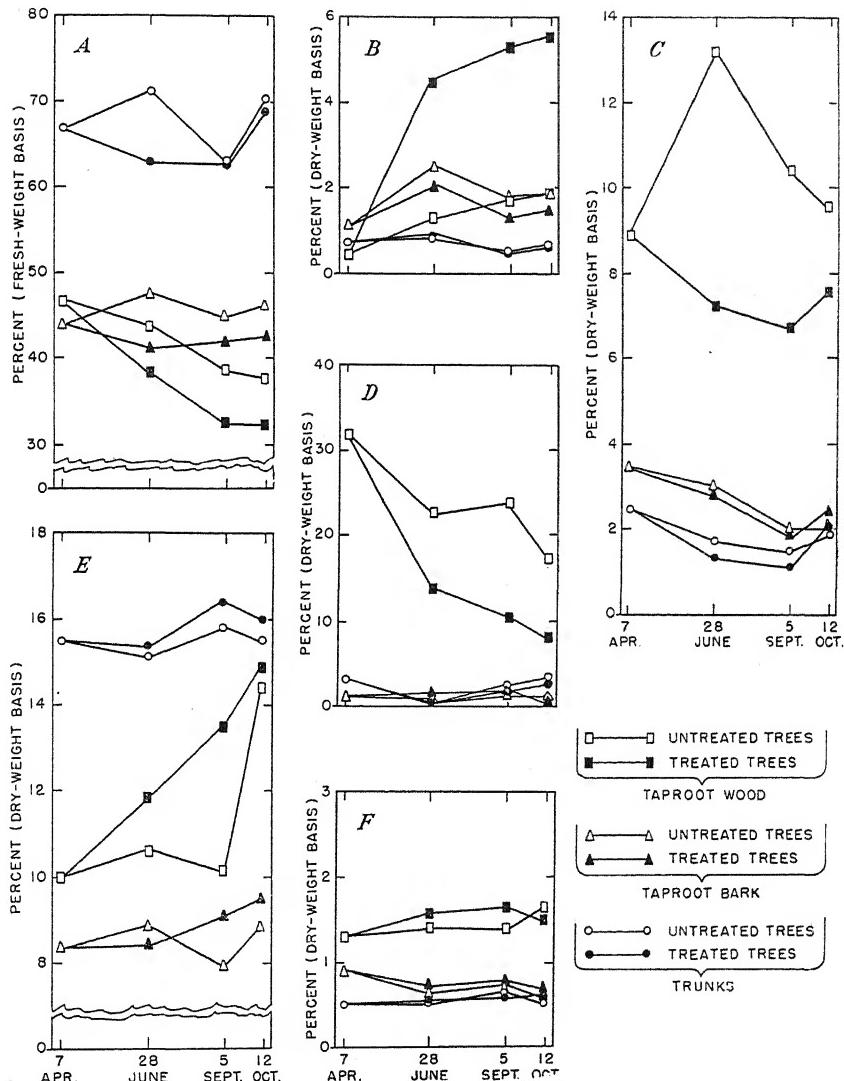


FIGURE 1.—Seasonal changes in dry matter (A), reducing sugars (B), nonreducing sugars (C), starch (D), hemicellulose (E), and organic nitrogen (F) in untreated (normal) pecan trees and in similar ones whose taproots received an application of 8 mg. of indole-3-butyric acid prior to transplanting, 1944.

treated seedlings, however, were much smaller than those in that of the untreated, which showed a sharp increase from April 7 to June 28 and a similar decrease to October 12. The increase on June 28 was in sharp contrast to the decrease in treated trees. At the end of the experiment the taproot wood of untreated trees contained more non-reducing sugars than did that of the treated trees.

#### STARCH

The treatment apparently had little effect on the low percentages of starch in the trunks and taproot bark (fig. 1, D). On the contrary, it caused a considerable reduction in the high starch content of the taproot wood; during the season the percentages decreased from 31.89 to 8.15 and 17.30, respectively, in the treated and the untreated trees.

#### HEMICELLULOSE

The percentages of hemicellulose in the trunks were not appreciably affected by the treatment (fig. 1, E). In the taproot wood of treated trees the percentage increased rapidly throughout the experiment in contrast to that in untreated trees, which fluctuated slightly from April 7 to September 5, and then increased very sharply to October 12, when it was almost as high as that in treated trees. Although the percentages fluctuated somewhat differently in the taproot bark of treated and untreated trees, it is doubtful whether the hemicellulose content was affected by the treatment.

#### ORGANIC NITROGEN

The curves for percentages of organic nitrogen in the trunks and taproot bark of treated and untreated trees differed little and fluctuated similarly (fig. 1, F). In the taproot wood of treated trees the percentages increased appreciably from April 7 to September 5, but they remained almost constant in that of untreated trees. Because of opposite trends during the last period of the experiment the final percentages were about equal.

#### EFFECT OF TREATMENT ON GROWTH

At each sampling date the new root and shoot growth was much greater in treated trees than in similar untreated ones (table 1). The

TABLE 1.—*Effect of indolc-3-butyric acid treatment of taproots on the growth of transplanted, nursery-grown, 4- to 6-year-old pecan trees, 1944*

[Treatment, 8 mg. per taproot; each value based on 14 trees]

Sampling date and status of trees	Average dry weight per tree of—	
	New roots	New shoots and leaves
June 28:		
Treated	Grams 2.07	Grams 6.54
Untreated	.04	3.91
September 5:		
Treated	t.92	12.74
Untreated	.55	8.43
October 12:		
Treated	4.20	24.10
Untreated	.54	12.07

ratio of new root growth of treated to that of untreated trees was, however, much greater than the similar ratio for growth of new shoots and leaves. This indicates that indole-3-butyric acid stimulates the initiation and growth of roots to a greater extent than it does shoots and leaves.

#### INTERRELATION OF CHANGES IN COMPOSITION

In a comparison of the changes in percentage composition of the different tissues of treated and untreated pecan trees during the 1944 season, it was found that in the majority of cases where changes occurred they were in the same direction but not to the same extent. The main exception was in the percentage of nonreducing sugars of taproot wood, where the changes in treated trees were in the reverse direction from those in untreated ones. As a general rule greater changes occurred in the percentage composition of tissues of treated trees than in those of untreated; the former also made more new root and top growth, and therefore greater changes in composition were to be expected.

In both treated and untreated trees most of the elaborated food materials available for growth functions during the experiment were those stored at the time of transplanting, because the trees had small root spreads for absorption of soil nutrients and produced few leaves for photosynthetic processes, particularly during the first part of the experiment. Thus, it is not surprising that the main changes in percentage composition in the tissues analyzed were centered around those in starch content, especially in the taproot wood. The highest starch content was found in taproot wood; the greatest changes in percentage composition and the greatest difference between the changes in treated and untreated trees occurred in this tissue. The starch content of taproot wood was high at the beginning of the experiment and the percentages decreased greatly during the season, but the decrease was about 1.6 times as great in treated trees as in those not treated.

The percentage of dry matter in taproot wood of treated trees also decreased to a much greater extent than did that in untreated ones. The percentages of reducing sugars were much higher in taproot wood of treated trees than in that of untreated ones, but the reverse was true of nonreducing sugars; consequently, the differences in percentages of total sugars in taproot wood of treated and untreated trees were not great.

The percentage of hemicellulose in taproot wood of treated trees increased rapidly throughout the experiment, whereas in that of untreated ones the changes were relatively small until the final stage of the experiment, when the percentage increased sharply. The increases in percentages of hemicellulose in taproot wood, as well as the variations in percentages of the other constituents, may be largely accounted for by the decreases in the percentages of starch. The starch percentages decreased very rapidly, indicating an actual decrease in starch content. Since starch made up almost one-third of the dry matter of taproot wood at the beginning of the experiment, a rapid decrease in its percentage (dry-weight basis) would mean a corresponding decrease in percentage of dry matter; this is what occurred. Therefore, if there were little or no change in the actual amount of hemicellulose

in taproot wood during the experiment, the percentages (dry-weight basis) would increase in proportion to the decrease in starch or other dry matter; in fact, the actual amounts of hemicellulose could decrease under such conditions while the percentages increased for the same reason.

It is likely that there were actual decreases in nonreducing sugars in all of the analyzed tissues of treated trees, since the percentages on the dry-weight basis decreased during the experiment.

#### SUMMARY AND CONCLUSION

The taproots of nursery-grown pecan seedlings were treated with approximately 8 mg. of indole-3-butyric acid per tree at the time of transplanting, and the percentages of sugars, starch, hemicellulose, organic nitrogen, and dry matter of the taproot wood, the taproot bark, and the trunks at intervals during the first growing season were compared with those for the tissues of similar untreated trees.

As a whole, the important changes in composition of the trees may be centered around the decreases in starch content, particularly in the taproot wood, in which the percentage of starch was very high (31.89 percent of dry matter) at the beginning of the experiment and the decrease during the season was 1.6 times as great in treated trees as in untreated ones. The decreases in starch content would affect the percentages of other materials such as sugars and hemicellulose (dry-weight basis) and dry matter (fresh-weight basis) in proportion to the decreases in starch. Considerable changes occurred in percentages of nonreducing sugars, but it would appear that changes in the labile forms of carbohydrates are of less value in a study of the effects of the indole-3-butyric acid than are changes in amounts of starch from which they probably were derived, since the equilibrium levels of the labile forms may fluctuate more than those of starch.

There were changes in the composition of the trunks and the taproot bark, but they were largely in the same direction in treated and untreated trees, and the differences can probably be explained on the same basis as the changes in composition of taproot wood.

The treated trees made more new root and shoot growth than untreated ones; as would be expected there were greater changes in the chemical composition since the trees were largely dependent on stored nutrient materials to support growth functions during the period of the experiment.

It is therefore apparent that a relatively small quantity of indole-3-butyric acid, when applied to taproots of transplanted pecan trees, stimulates the processes of initiation and growth of new roots and of top growth; as a consequence greater amounts of the stored nutrient materials are used up during the first growing season than in similar untreated trees.

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## ACIDIC AND PHENOLIC FRACTIONS OF CRUCIFER ROOTS IN RELATION TO CLUBROOT<sup>1</sup>

By FREDERICK G. SMITH, formerly research associate in biochemistry and plant pathology, KARL PAUL LINK, professor of biochemistry, and J. C. WALKER, professor of plant pathology, Wisconsin Agricultural Experiment Station<sup>2</sup>

### INTRODUCTION

One phase of a program of study of resistance in crucifers to the clubroot disease (*Plasmodiophora brassicae* Wor.) (17, 20, 21, 25)<sup>3</sup> has been the biochemical investigation of the role of fungicidal constituents of the host plant. When Stahmann, Link, and Walker (20) found no evidence that either the nature or quantity of the mustard oils or the activity of myrosin, the mustard-oil glycosidase, was responsible for resistance, attention was directed toward the possible role of other types of compounds in the tissue which might contribute to resistance. The present paper presents the results of a study to determine the relation of the acidic and phenolic constituents of the root tissues to the resistance of crucifers to clubroot infection.

Acidic and phenolic compounds have received the attention of other workers in disease resistance. Cook and Taubehaus (2, 3) and others (4, 11)<sup>4</sup> suggested that tannins or similar conjugated phenolic compounds were active in disease resistance. Dufrénoy (6) claimed that phenols were characteristic products of the cells of invaded tissues and that they might inhibit the spread of the pathogen. Kargapolova (12) reported that resistance in wheat to *Puccinia triticina* Eriks. was associated with special types of phenolic compounds in particular varieties. In studies of wheat rust (*Puccinia graminis* Pers.) by Newton and Anderson (15) and of sugarcane red rot (*Physalospora tucumanensis* Speg.) by Abbot (1), colorimetric estimates of phenols were reported to correlate with resistance. In only one case, however, has it been shown unequivocally that resistance is due to specific phenolic compounds. Walker, Link, and associates (13, 14, 23) showed that protocatechuic acid (3, 4-dihydroxybenzoic acid) and catechol (1, 2-dihydroxybenzene) were largely responsible for the resistance of pigmented onions to *Colletotrichum circinans* (Berk.) Vogl., the smudge organism. Other more strongly acidic compounds, such as carboxylic acids, have also been considered in resistance studies. Investigation of root rot (*Phymatotrichum omnivorum* (Shear) Dugg.) of cotton in Texas (7, 9, 18) has included the relation of acidic constituents of monocotyledonous plants to resistance as well as the effect of various acids and phenols on the causal organism itself.

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<sup>2</sup> The writers are indebted to Mark A. Stahmann for advice during this investigation.

<sup>3</sup> Numbers in parentheses refer to Literature Cited, p. 203.

<sup>4</sup> OFFORD, H. R. THE FUNCTION OF TANNIN IN HOST-PARASITE RELATIONSHIPS WITH SPECIAL REFERENCE TO RIBES AND CRONARTIUM RIBICOLA. U. S. Dept. Agr. Bur. Ent. and Plant Quar. E-518. 27 pp. 1940. [Processed.]

Preliminary work on various acidic fractions suggested two lines of approach. The first and most important was to determine whether there was a significant difference in fungicidal activity between extracts of resistant and susceptible turnip roots. The second was to determine whether the concentration of phenols, estimated colorimetrically, was correlated with resistance. These must necessarily have preceded any extensive efforts to isolate and identify the toxic materials. Both lines of approach were based on rather crude measurements, neither of which had any specific relation to the clubroot disease. Accordingly, to associate resistance clearly with such materials it was necessary, first, to demonstrate marked differences between resistant and susceptible turnip varieties in toxicity or in phenol content, and then to show that similar differences usually existed between resistant and susceptible varieties and species of the Cruciferae.

#### EXPERIMENTAL RESULTS RESISTANT AND SUSCEPTIBLE HOSTS

Turnip (*Brassica rapa* L.) varieties previously tested by Walker (21) for resistance or susceptibility to clubroot were used as the source of plant extracts. In the beginning, the variety Purple Top Milan was selected as the resistant type inasmuch as it was shown to remain completely free from infection when exposed in a number of trials to the Wisconsin collection of the pathogen. This variety will be referred to hereafter in this paper as "R." Variety Shogoin was chosen as the susceptible host type because it consistently showed a high percentage of infected plants when inoculated with the pathogen. This variety will be referred to as "S." Later work was extended to other varieties of turnip and other crucifers, selected on the basis of their known resistance to the pathogen. Extra Early White Milan and Snowball turnip varieties were used as completely resistant varieties; Cowhorn was chosen as one which showed 25 to 40 percent susceptible individuals in the population (21).

Cabbage (*Brassica oleracea* var. *capitata* L.) was used as representative of very susceptible crucifers. A strain of rape kale (*B. napus* L.) found in numerous tests to be highly resistant was included. Two strains of black mustard (*B. nigra* L. Koch), the same as used by Pryor (17), were in one case highly resistant and in the other very susceptible to clubroot.

#### METHODS OF TOXICITY TESTING

Since toxicity to *Plasmodiophora brassicae* could not be conveniently or accurately measured, fungicidal activity was determined on *Colletotrichum circinans*. This organism had been used previously in testing the toxicity of phenols and mustard oils (22, 24) and was shown to have sensitivity to the latter similar to that of *P. brassicae* (10). Two widely used types of assay were employed, the first based on the effect of toxicant on the rate of growth of the test organism, the second on the extent of germination of the spores.

The first type of assay used in the preliminary trials was similar to the technique of earlier work in this series (22, 24) except that volumes were scaled down to 10 ml. per tube and observations were based on visual examination of growth rather than on mycelial weights. This technique had several disadvantages. It required 20 to 30 ml. of sample representing 200 to 300 gm. of fresh tissue and con-

siderable labor to set up, and it took several days to determine the results.

For these reasons the second type of test was developed, which was more convenient and economical in time and material (19). This technique was used routinely in all experiments reported here but was compared directly with the growth test on several typical extracts to make possible comparisons with earlier work. The following set of indices was used to express the results of the germination tests.

The inhibitory concentration, "IC," is that least concentration (grams dry weight per milliliter solution) of extract which caused complete (or nearly complete) inhibition of germination when the next lower concentration (in steps of one-half) allowed nearly complete germination. When the range from about 0 percent to about 100 percent germination was more than one concentration step, the IC was not considered fully reliable.

The toxicity level, "TL," which was equal to  $1/IC$ , was thus a rough measure of the concentration of toxic substances in the fraction being tested. "TR" refers to the quotient of the TL of the resistant tissue extract divided by the TL of the susceptible tissue extract.

In experiments on methods of extraction and fractionation corresponding R and S fractions originally were compared as they occurred with the assumption that any differences in composition which caused differences in toxicity might be important in resistance. Consequently, no adjustment of pH, for example, was made. When it was later shown, however, that the concentration of strong acids altered toxicity of those fractions by shifting the pH (19), it was necessary to modify this assumption. Toxic substances effective in disease resistance *in vivo* would presumably be acting in the normal pH range of host cells, so it was decided to adjust all extracts to a uniform pH of 5.5 to 6.0. This cut down toxicity of most fractions but better approximated the conditions of host-parasite interaction in disease. The revised technique was used in the final extended analysis of tissues of a number of crucifers.

#### COLORIMETRIC METHODS FOR PHENOLS

It became evident early that several types of phenols, including the more labile polyphenols, occurred in turnip extracts but in relatively low concentration. It was necessary, therefore, to select analytical methods which were as general as possible without serious loss of specificity, were of maximum sensitivity, and were satisfactory for the labile polyphenols (ortho- and paradihydroxy-phenols). Folin's phosphomolybdic-phosphotungstic acid method and the diazo methods are the two most widely used colorimetric methods. However, they have been used largely with mono- and metadihydroxy-phenols in animal tissues or in nonbiological materials, where purification is limited to precipitation of proteins or other nitrogenous-reducing compounds or to distillation of the phenols themselves. With plant material Newton and Anderson (15), employing the Folin method, used an ether-soluble, alkali-soluble fraction to avoid interfering compounds. They did not consider, however, the effect of alkaline extraction or the technique of color development on the labile polyphenols. The diazo methods are somewhat less general than the Folin method but because of their greater specificity can often be used with less purification of extracts.

Preliminary studies of the Folin and diazo colorimetric methods, which cannot be reported in detail, showed that neither was altogether satisfactory for the labile polyphenols. Alkaline extraction and small variations in the technique of color development caused marked changes in color values by both methods. The best results were obtained with the techniques described below, based on those of Folin and Ciocalteu (8) and Deichmann and Scott (5), when special care was exercised to maintain uniform conditions in the fractionation and color development.

*Folin technique:* Suitable volumes of an aqueous solution containing 1 to 5 mM. (micromoles) of phenols were diluted to 20 milliliters. One milliliter of Folin-Ciocalteu reagent was added and the solution stirred. Five milliliters of 20 percent sodium carbonate was then quickly added and the solution again stirred. Finally, the solution was allowed to stand 30 minutes and the color read against a phenol standard in a Duboscq colorimeter. This standard containing 0.100 mM. of phenol per milliliter was prepared daily from a stock solution of 1.00 mM. of phenol per milliliter in 0.01 M sulfuric acid.

*Diazo technique:* The aqueous solution was neutralized with dilute sodium hydroxide to pH 6.5 to 7.0, or low enough to destroy the buffering capacity of the acids and permit the sodium acetate to raise it into this range. A suitable volume of solution to contain 0.25 to 2.0 mM. of phenol was diluted to 20 ml., and 4 ml. of 25 percent sodium acetate containing 0.5 percent of gum acacia was added with stirring. Then 2 ml. of diazo reagent was added, the solution mixed and allowed to stand 1 minute, and 4 ml. of 20 percent sodium carbonate added with stirring. After 3 minutes the color was compared with a phenol standard as in the Folin technique. It was usually found necessary to make corrections for appreciable color in the controls. This color error was traced to the gum acacia but could not, however, be completely eliminated by several precipitations of the gum from alcohol-water mixtures. The error was compensated as indicated in the following formula:

$$Px = RxPs \left( \frac{1+Rc}{1-Rc} \right)$$

Where  $Px$ =true phenol content of sample;

$Rx = \frac{Px}{Ps + P_c}$  =colorimeter reading of sample against standard;

$Ps$ =true phenol content of standard;

$P_c$ =apparent phenol content of water control;

$Rc = \frac{P_c}{Ps + P_c}$  =colorimeter reading of water control against standard.

This correction often changed final values by 10 to 20 percent.

The phenol level, "PL," was expressed in micromoles per gram of fresh tissue. "PR" was the PL of resistant root extract divided by the PL of susceptible root extract.

The acidity level, "AL," was the titrable acidity in microequivalents per gram of fresh tissue. "AR" was AL of resistant root extract divided by the AL of susceptible root extract.

#### APPLICATION OF PHENOL METHODS TO FRESH TISSUE

Concurrently with the toxicant-fractionation experiments the phenol content of R and S fresh tissue was investigated by using the

two colorimetric methods and a fractionation scheme similar to Newton and Anderson's (15).

The first attempts were to measure only sap-soluble phenols, avoiding enzymatic or other hydrolytic action as far as possible. Fifty grams of the 2-mm. outer peeling of fleshy roots, consisting largely of periderm, phloem, and secondary xylem, were sliced, rapidly frozen with powdered dry ice, and the cold juice, expelled at 2,000 pounds per square inch, was immediately sucked into 25 percent trichloroacetic acid. The press cake was washed twice by soaking in the same solvent and repressing and the combined aqueous solution extracted with ether continuously in a liquid-liquid extractor. Comparison of apparent phenol content of press juice and ether extract by the diazo method showed that only 5 to 10 percent of the apparent phenols was extracted by ether so that further fractionation was necessary with both colorimetric methods. The ether concentrate was then extracted with 5 percent NaOH to give the "acidic-amphoteric fraction" which was acidified and again extracted with ether to give the "acidic fraction." Reproducibility and recovery of added phenols by this technique was only fair because of anomalous effects of alkaline extraction and occasional turbidity of final aqueous solutions. The PR ratio, the most reliable measure of relative phenol content of the two tissues, was generally greater than 1 but never significantly large (table 1).

TABLE 1.—*Phenol content of various fractions of fresh-tissue extracts from resistant (R) and susceptible (S) turnip root*

Extraction series Number	Source of extract	Ether-soluble fraction				Acidic-amphoteric fraction		Acidic fraction			
		Diazo method		Folin method		Diazo method		Diazo method		Folin method	
		PL <sup>1</sup>	PR <sup>2</sup>	PL <sup>1</sup>	PR <sup>2</sup>	PL <sup>1</sup>	PR <sup>2</sup>	PL <sup>1</sup>	PR <sup>2</sup>	PL <sup>1</sup>	PR <sup>2</sup>
3	{R. S.	0.13 .08	1.6	-----	-----	{ 0.11 .07	1.6	-----	-----	-----	-----
5	{R. S.	.12 .085	1.4	-----	-----	{ .12 .10	1.3	{ 0.10 .10	1.0	-----	-----
7	{R. S.	.15 .09	1.7	{ 0.28 .22	1.3	{ .10 .08	1.3	{ .04 .05	.8	{ 0.19 .16	1.2
8	{R. S.	-----	-----	-----	-----	-----	-----	{ .07 .05	1.4	{ .23 .22	1.0
8A <sup>3</sup>	{R. S.	-----	-----	-----	-----	-----	-----	{ .13 .16	.8	{ .20 .22	.9
9A <sup>4</sup>	{R. S.	-----	-----	-----	-----	-----	-----	{ .22 .24	.9	{ .47 .41	1.1

<sup>1</sup> PL=phenol level as micrograms of phenol per gram of fresh weight of tissue.

<sup>2</sup> PR=PL of R tissue divided by PL of S tissue.

<sup>3</sup> Extraction of press cake from series 8.      <sup>4</sup> Autolyzed.

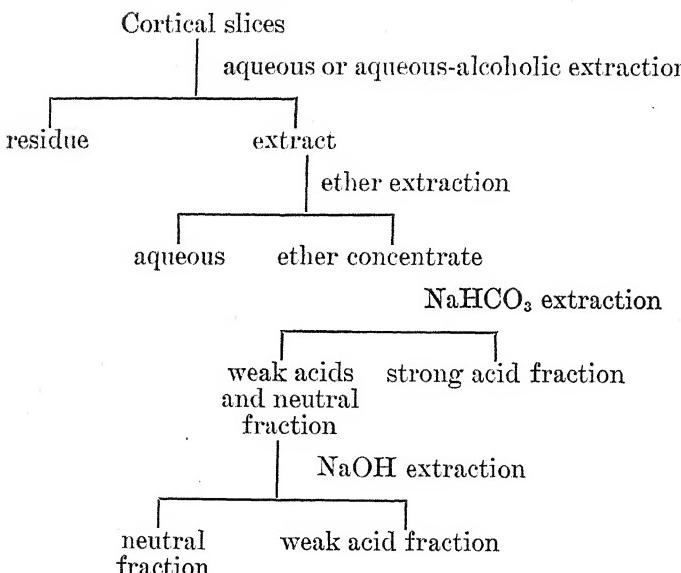
The lack of large differences between R and S in phenol content of press-juice fraction and previous experience with acid extractant suggested that greater differences might be found in unextracted or bound forms of phenols. Exhaustive alcohol extraction of the press cake showed that the major part of the tissue phenols was not in the press juice but could be removed by more thorough extraction. Fractionation and analysis of these extracts, however, showed no difference between R and S (table 1). Acid hydrolysis of press juice also failed

to give greater differences though there was a marked increase in phenol levels. Autolysis was also tried in a further effort to demonstrate differences in phenol content which might arise from hydrolytic or oxidative processes similar to those observed by Parker-Rhodes (16) with host extracts and rust uredospores. Tissue slices were frozen in dry ice and allowed to thaw and autolyze at room temperature for 48 hours before pressing and fractionating. Typical results in table 1 showed an increase in phenol concentrations as in the case of acid hydrolysis but no difference in PL's of R and S tissues.

These results of colorimetric analysis of phenols in fresh tissue indicated (1) no significant differences in press juice fractions and (2) increased yield without increased differences by alcohol extraction, acid hydrolysis, and autolysis.

#### EXTRACTION AND FRACTIONATION

An extensive series of experiments which will not be reported in detail was carried out to determine the effect of various methods of extraction and fractionation on the relative fungicidal activities of R and S tissues. Aqueous and 30 percent alcoholic extracts of dried cortical tissues, both neutral and acid, from 1938 and 1939 crops were fractionated by the following scheme.



The results in brief were (1) PR's were consistently greater than 1 only in the strong acid fractions, but the differences were never in proportion to the differences in disease resistance; (2) acid extraction under mild conditions gave higher PL's and higher PR's than neutral conditions, while more drastic acid conditions diminished the PR's; (3) alcoholic extraction cut down the interference of pectic materials in the ether extraction step but gave no higher yield of fungicidal materials.

The technique of extraction and fractionation finally adopted for extended analysis of crucifer tissue and designated as the PT method

was as follows. Sliced, fresh cortical peelings in 500-gm. lots were refluxed in quart mason jars for 30 minutes in an aqueous solution of 0.5 percent sulfuric acid and 45 percent alcohol (including tissue water). After 8 months' storage at room temperature each lot was filtered, homogenized in a Waring blender with fresh solvent, and the pulp squeezed in a hand screw press. The combined alcoholic extracts were concentrated *in vacuo* at 25° to 30° C. to about 100 ml., diluted with water, and reconcentrated twice to remove all alcohol. The resulting aqueous suspension was cooled to 6° C. overnight and filtered through a mat of Filter Cel to remove as much of the gummy, water-insoluble material as possible. The filtrates were then diluted to about 800 ml. and extracted with ether for 36 hours in a continuous liquid-liquid extractor. The ether extracts were made up to 200 ml. and shaken six times with 100 ml. of 5 percent sodium bicarbonate for 4 minutes, each extract being run quickly into excess dilute sulfuric acid. These acidified solutions were again extracted continuously with ether for 24 hours and the resulting "strong acid fraction" dried over sulfuric acid, concentrated to 50 ml., and stored at 6° C.

Limited application of the PT method to R and S tissue of the 1940 crop was made and germination tests, phenol analyses, and acidity titrations were run on aqueous solutions made by suspending aliquots of the ether concentrates in water and evaporating the ether. Titration curves of various lots were found to be similar, so titratable acidity was measured as the micro equivalents of acid per gram of fresh tissue titrated to an equivalence point of about pH 8. In several cases large differences in fungicidal activity, in phenol content, and in titratable acidity were found between R and S tissue (table 2).

TABLE 2.—*Typical results of strong acid fractions from extraction and fractionation of R and S tissues by the PT method*

Series No.	Source of extract	Fungicidal activity	Phenol content				Titratable acidity	
			Diazo method	Folin method	PL <sup>1</sup>	PR <sup>1</sup>		
PT2.....	{R S.....	TL <sup>1</sup> 16-29 2 } TR <sup>1</sup> 8-16	{ 1.3 .35 }	3.8	{ 1.1 .36 }	3.2	{ 16.3 .74 }	22
PT4.....	{R S.....	32 2-4 }	8+{ .13 .026 }	5	{ .48 .10 }	5	{ 11.5 .37 }	30

<sup>1</sup> For explanation of symbols, see Methods of Toxicity Testing, p. 194, and Colorimetric Methods for Phenols, p. 195.

In assaying these particular strong-acid fractions it was also observed for the first time that the fungicidal activity varied markedly with the pH of the test solution. Toxicity of these fractions increased with increasing hydrogen-ion concentration from pH 6 to 3, its behavior in this respect being similar to that of carboxylic acids (19). In fractionation these acids had been separated from the natural buffers of the tissue, and in the unbuffered test solutions they were acting at pH values considerably below the physiological range of the host tissues. Thus strong acids by lowering the pH exerted a misleading indirect effect on the fungicidal activity of any compounds, such as carboxylic acids and phenols, whose toxicity varied with hydrogen-ion concentration. For this reason all subsequent toxicity assays were made in buffered solutions.

## EXTENDED ANALYSIS OF CRUCIFER EXTRACTS BY THE PT METHOD

An extensive test of the methods of the previous section was carried out on a new lot of tissue grown in the summer of 1941 along with some of the 1940 lot. Cabbage and rape, kale, several varieties of turnip, including the R and S varieties, and a resistant and a susceptible strain of mustard were preserved by the PT method, both in neutral alcohol and acid alcohol containing 0.5 percent sulfuric acid. Several samples of each variety and each treatment were fractionated and strong-acid and weak-acid fractions prepared.

Phenol analyses were run two to four times on each of two aliquots of the ether concentrates. The analyses were carried out in large numbers so that many samples could be compared directly with each other without depending upon the less accurate comparison of extracts with pure phenol standards. Different runs were, however, all calculated to a phenol basis. This method of comparing different aliquots of the same ether concentrate or of the same aliquot analyzed at different times showed variations of less than 10 percent, which was considered definitely less than the errors due to fractionation of and variation in tissue. Toxicity measurements were carried out by the revised germination technique eliminating the pH effect and were expressed as means of two to four separate tests.

TABLE 3.—*Summary of analyses by the PT method and toxicity tests of several crucifers*

Plant species and variety	Resistant or susceptible	Year sample was grown	Type of fraction	Experiment No.	Phenol content (PL) <sup>1</sup>		Titratable acidity (AL) <sup>1</sup>	Toxicity (TL) <sup>1</sup>
					Diazo method	Folin method		
Turnip (var. Purple Top Milan).	Resistant	1940	Acid-alcohol; strong acid.	7A-----	0.23	0.69	20.0	2-4
		1940	do	7B-----	.21	.68	21.0	4-
		1940	do	9A-----	.059	.020	.32	4
		1940	do	9B-----	.033	.11	.82	1-2
		1941	do	9C-----	.21	.60	16.6	2
		1941	do	10B-----	.23	.75	19.5	2-4
		1941	Neutral-alcohol; strong acid.	11A-----	.088	.24	.65	-----
		1941	do	11B-----	.14	.32	.65	2-
		1941	Acid-alcohol; weak acid.	10A-----	.011	.04	-----	-----
		1941	do	7A-----	.014	.054	-----	-----
		1941	Acid-alcohol; strong acid.	4bA-----	.17	.64	13.0	-----
		1940	do	4dA-----	.19	.20	.32	2-4
Turnip (var. Shogoin)..	Susceptible	1940	do	4dB-----	.19	.55	14.6	2
		1941	do	13A-----	.066	.23	6.5	-----
		1941	do	15A-----	.10	.32	7.8	2
		1941	Neutral-alcohol; strong acid.	10A-----	.079	.31	-----	2
		1941	do	10B-----	.11	.29	1.3	2+
		1941	do	12A-----	.019	.042	.26	-----
		1941	do	4dB-----	.023	.082	-----	-----
		1941	Acid-alcohol	25A-----	.19	.64	16.1	2
Turnip (var. Cowhorn).	Partially resistant	1941	strong acid.	25B-----	.32	1.09	9.6	4-
		1941	do	24A-----	.091	.34	.91	-----
		1941	Neutral-alcohol; strong acid.	24B-----	.080	.22	.97	-----
		1941	do	25A-----	.017	.071	-----	-----
Turnip (var. Extra Early White Milan).	Resistant	1941	Acid-alcohol; strong acid.	21B-----	.27	.80	18.0	4
		1941	do	21C-----	.31	.98	19.5	4
		1941	Neutral-alcohol; strong acid.	20A-----	.17	.43	.53	-----
		1941	do	20C-----	.09	.25	-----	-----

See footnotes at end of table.

TABLE 3.—Summary of analyses by the PT method and toxicity tests of several crucifers—Continued

Plant species and variety	Resistant or susceptible	Year sample was grown	Type of fraction	Experiment No.	Phenol content (P%) <sup>1</sup>		Titratable acidity (AL)	Toxicity (TL) <sup>2</sup>
					Dianzo method	Folin method		
Turnip (var. Snowball).	Resistant	1941	Acid-alcohol; strong acid.	23A	0.35	1.16	15.6	2-4
		1941	do	23B	.29	1.05	14.8	2-4
		1941	do	23C	.32	.84	14.6	-----
		1941	Neutral-alcohol; strong acid.	22A	.073	.18	.97	-----
		1941	do	22B	.084	.25	.97	-----
		1941	Acid-alcohol; weak acid.	22B	.017	.041	-----	-----
Rape kale.....	Resistant	1941	Acid-alcohol; strong acid.	31A	.25	.93	13.9	4-
		1941	do	31B	.27	1.04	11.0	-----
Cabbage.....	Susceptible	1941	Acid-alcohol; strong acid.	33A	.19	.56	5.06	2
		1941	do	33B	.16	.49	-----	-----
Black mustard.....	Resistant	1941	Acid-alcohol; strong acid.	-----	.33	1.13	14.5	1-2
	Susceptible	1941	do	-----	.25	.91	13.7	2-

<sup>1</sup> For explanation of symbols see Methods of Toxicity Testing, p. 194, Colorimetric Methods For Phenols, p. 195.

The results summarized in table 3 show no significant correlation between IC's and resistance—nearly all assays lay within the narrow range of 2 to 4. Buffering the test solutions to eliminate the pH effect had markedly reduced the TL's as expected. Titratable acidity, likewise, showed no clear correlation with resistance though there was fair agreement among replicates, with a few exceptions. These exceptions were acid-alcohol, strong-acid fractions whose AL's dropped into the range of the neutral-alcohol fractions. Whether this was due to failure of the 0.5 percent sulfuric acid in these cases to exert its usual effect on strong-acid extraction or to tissue variation could not be determined. The large differences in phenol content between resistant and susceptible host tissues of the 1940 trials were also not confirmed and considerable variation among replicates was apparent. A few replicates consistently fell below others in the treatment, somewhat as in the case of titratable acidity, showing, in fact, a fair correlation between phenol and strong-acid content. It must be assumed that these represent either tissue variation or some unknown discrepancy in the fractionation. Disregarding such samples, however, would not alter the over-all interpretation of the results. Neutral extraction again gave variable but generally lower phenol content than acid extraction. Representative weak-acid fractions showed usually less than one-tenth the phenol content of corresponding strong-acid fractions. The major phenolic compounds of these crucifer extracts were apparently strong enough acids to extract into the sodium-bicarbonate-soluble acid fraction.

#### DISCUSSION

Previous work in this laboratory had suggested that other fungicidal constituents of crucifers than the mustard oils might be involved in resistance to clubroot and certain evidence pointed to acidic or

phenolic fractions. The first objective of the present work, accordingly, was to determine whether there was a significant correlation between fungicidal activity of extracts or particular fractions and the resistance of crucifers in the field. An attempt was made at the same time to determine whether phenolic compounds, specifically, were involved by colorimetric estimation in several types of crucifer extracts.

Study of the effect of extraction and fractionation methods on toxicity confirmed preliminary evidence that the ether-soluble, strong-acid fraction showed the most consistent differences in toxicity between extracts from resistant and susceptible turnips. Acid extraction or hydrolysis tended to increase toxicity levels of extracts of both types of tissue and under certain conditions seemed to increase differences between them. In fact, one method involving acid extractants (PT method) with turnip tissue of the 1940 crop showed very significant differences in fungicidal activity, phenol content, and titrable acidity. More important, however, these experiments revealed that the titrable acidity of acid fractions varied considerably in different tissue samples and caused marked variation in the pH of extracts used in toxicity testing. Study of the influence of pH on the toxicity of turnip extracts (19), moreover, showed that the greater part of the toxicity of unbuffered extracts was probably due to this increased fungicidal activity of strong acids at low pH values.

More extensive analyses of several crucifers from the 1941 crop showed no significant correlation between toxicity and resistance. It seemed probable that the several cases of large differences in toxicity of extracts of resistant and susceptible turnips in preliminary trials were due mainly to differences in acid content. With the pH effect of acids on toxicity eliminated it is doubtful whether such correlation would ever have appeared.

Application of Newton and Anderson's method of extraction of phenols by using both the Folin and diazo colorimetric methods on fresh turnip tissues showed a possible correlation of sap-soluble phenol content with resistance. It was found, however, that the major part of the extractable phenols was freed only by acid hydrolysis or autolysis. These methods, however, still produced no clean-cut correlation of phenol content and resistance. The phenol levels found were close to those from autolyzed fresh tissue and the ratio of levels by the Folin and diazo techniques was similar to those in press-juice fractions. It should also be pointed out that the average concentration of phenols found in crucifer roots was somewhat less than that reported by Newton and Anderson (15) in wheat seedlings and probably less than one-tenth the amounts shown by Link and associates (13, 14) to be present in the outer scales of onions and to be effective in resistance to smudge. The correlation of phenol content in crucifer roots with clubroot resistance is not as strong as in the case of onion smudge (13, 14) and the relatively low concentrations present might argue against any relation with disease resistance. As pointed out in another paper (19), however, small differences in hydrogen-ion concentration of resistant and susceptible tissue may cause relatively large differences in the fungicidal effectiveness of certain cellular constituents. Although present evidence fails to demonstrate that resistance to clubroot in crucifers is due in any large part to readily extractable phenolic or acidic toxic constituents of the root tissues, the possibility

still remains that fungicidal compounds are produced during invasion of the pathogen in response to special stimuli or are caused by derangement of normal metabolism. The methods employed in the present investigation would hardly have detected mechanisms of this kind. Adequate study of such complex changes in invaded tissues with the possible production of fungicidal constituents will depend upon better understanding of the metabolism of normal tissues.

#### SUMMARY

The suspected significance of phenolic or acidic constituents of root tissues in resistance to clubroot of crucifers was investigated by phenol and acid analysis and toxicant fractionation.

Methods of phenol determination in plant tissue were studied, and modified Folin and diazo techniques were applied to the estimation of phenol content of fresh tissues. No clean-cut correlation was found between resistance and sap-soluble phenols of resistant (Purple Top Milan) and susceptible (Shogoin) turnip varieties. Exhaustive alcohol extraction, acid hydrolysis, and autolysis caused increased yields of phenols in both varieties but no significant differences.

Study of extraction and fractionation techniques confirmed the presence of toxic materials in the ether-soluble, sodium-bicarbonate-soluble fraction. These fractions were assayed for fungicidal activity by their effect on percentage germination or growth of *Colletotrichum circinans*. The most promising technique, the PT method, in limited application to the two turnip varieties of the 1940 crop, showed highly significant differences in phenol content, titrable acidity, and toxicity which correlated with resistance. It was discovered, however, that at least part of the toxicity of these fractions was due to the effect of strong acids on the pH of test solutions. Extensive application of the PT method to representative crucifers of varying field resistance from the 1941 crop and elimination of the pH effect in toxicity testing failed to show any clear correlation between phenol content, acidity, and fungicidal activity and resistance.

The results did not show that resistance to clubroot of crucifers was due in any large part to the presence of preformed extractable phenolic or acidic fungicidal constituents in the cortical tissues of the fleshy roots.

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# STUDIES ON THE INHERITANCE OF HARD SEEDS IN BEANS<sup>1</sup>

By G. A. LEBEDEFF

*Associate agronomist, Georgia Agricultural Experiment Station*

## INTRODUCTION

The presence of hard seeds in many legumes is a serious problem to farmers, to seed dealers, to canners, and to consumers. In most States regulations for enforcement of seed laws specify tolerance of hard seed in various legumes. To reduce the number of hard seeds in small-seeded legumes, various methods of seed treatment, such as scarification or treatment with acids or hot water, have been applied. However, these practices are time consuming, expensive, and tend to mutilate the seeds. Bean canners have demanded that seedsmen eliminate hard seeds, and often require that the seed purchased be relatively low in moisture. To meet this latter requirement dealers dry seeds artificially, which tends to increase the number of hard seeds.

In previous studies<sup>2</sup> of the effect of heredity and environment on the production of hard seeds in beans (*Phaseolus vulgaris* L.), the seeds of 36 selections of common white beans were dried over calcium chloride to 10 different moisture contents, ranging from 14.11 to 5.59 percent. There were no hard seeds in the control which contained 15.14 percent of moisture, but lots of seed with 14.11 percent of moisture contained 1 percent of hard seeds. With each reduction in seed-moisture content the percentage of hard seeds increased and the seeds remained hard for a longer time. When moisture content was reduced to 5.59 percent nearly 90 percent of the seeds were hard.

Individual selections differed in regard to hard-seed production. Some selections developed a greater percentage of hard seeds in seed of the same moisture content; and in some selections hard seeds softened readily when tested for germination while in others they remained hard for a long time. These differences among individual selections in regard to hard-seed production were found to be hereditary.

In the present report the softening of hard seeds in the F<sub>1</sub> and F<sub>2</sub> generations of certain crosses is described.

<sup>1</sup> Received for publication May 10, 1945. Paper No. 139, Journal Series, Georgia Agricultural Experiment Station, Experiment, Ga.

<sup>2</sup> LEBEDEFF, G. A. HEREDITY AND ENVIRONMENT IN THE PRODUCTION OF HARD SEEDS IN COMMON BEANS (*PHASEOLUS VULGARIS*). Puerto Rico (Rio Piedras) Univ. Agr. Expt. Sta. Res. Bul. 4, 27 pp., illus. 1943.

## MATERIAL AND METHODS

Out of 36 selections studied previously, 6 selections, Nos. 12, 368, 475, 1083, 1099, and 1130, were chosen for further study. A detailed description of hard-seed production in these selections can be found in the publication referred to above.

Many crosses were made between these selections. The  $F_1$  plants, together with several plants of each parental selection, were grown in pots outdoors in partial shade during the spring of 1943 in Puerto Rico. The seeds were harvested about June 1 and stored in the laboratory. On August 15 these seeds, as well as extra seeds of the original crosses, were taken to Georgia where further studies were made under different environmental conditions and with modified methods.

Arrangements were made at Experiment, Ga., to grow the original 6 selections and the  $F_1$  and  $F_2$  plants from crosses between these selections at the same time and under similar conditions. Two greenhouses were available for this purpose. The seeds from crosses were planted on November 15 to grow the  $F_1$  plants. At the same time the seeds from 6 selections and those from the  $F_1$  plants were germinated in the laboratory (prior to germination the moisture content of this material was reduced to about 9 percent over  $\text{CaCl}_2$ ),<sup>3</sup> and the seedlings were transplanted to pots in the greenhouse. A total of 750  $F_2$  plants, over 100  $F_1$  plants, and over 100 plants of the 6 selections were grown in the greenhouses.

The seed from each plant was harvested separately during February 1944. All the threshing was done by hand, and the seeds from individual plants were put into individual cheesecloth bags and kept in the laboratory for about 3 months. Disregarding about 100 lots with less than 25 seeds which were discarded, the average number of seeds per plant was for the selections, 49.7; for the  $F_1$  plants, 53.3; and for the  $F_2$  plants, 45.2. Each curve in figures 1 to 5, therefore, is based on an average of about 50 seeds.

In order to induce hard-seed development in these 850 lots of seed, they were placed in an electric dehydrator of a cabinet type designed for home drying of fruit and vegetables.<sup>4</sup> The seeds, still in bags, were placed on seven trays inserted one above another in the drier. The trays were interchanged daily, and at the same time the bags were turned over to insure uniform drying. The temperature of the air in the drier remained approximately 45° C.

Several bags of seed were removed from the drier after 1, 2, and 3 days of drying and their moisture content was determined. After 5 days the drying was discontinued, and the seed lots, with the exception of 20 bags, were placed in an electric germinator. The moisture content of these 20 samples was determined, and this was taken to represent the approximate moisture content of the rest of this material. For determination of moisture content these 20 seed lots were crushed in a small meat chopper and then kept for 48 hours in an electric oven at 110° C. After the samples had been removed from the oven they were cooled in a desiccator and then weighed. The mean moisture content of the 20 samples was  $6.61 \pm 0.121$  ( $S. D. = 0.54 \pm 0.085$ ).

<sup>3</sup> Records were kept of hard-seed production in these seeds, but they are not reported here because more extensive data were obtained later.

<sup>4</sup> SHUEY, G. A. DEHYDRATION OF FRUITS AND VEGETABLES IN THE HOME. Tenn. Agr. Expt. Sta. Bul. 183, 21 pp., illus. 1943.

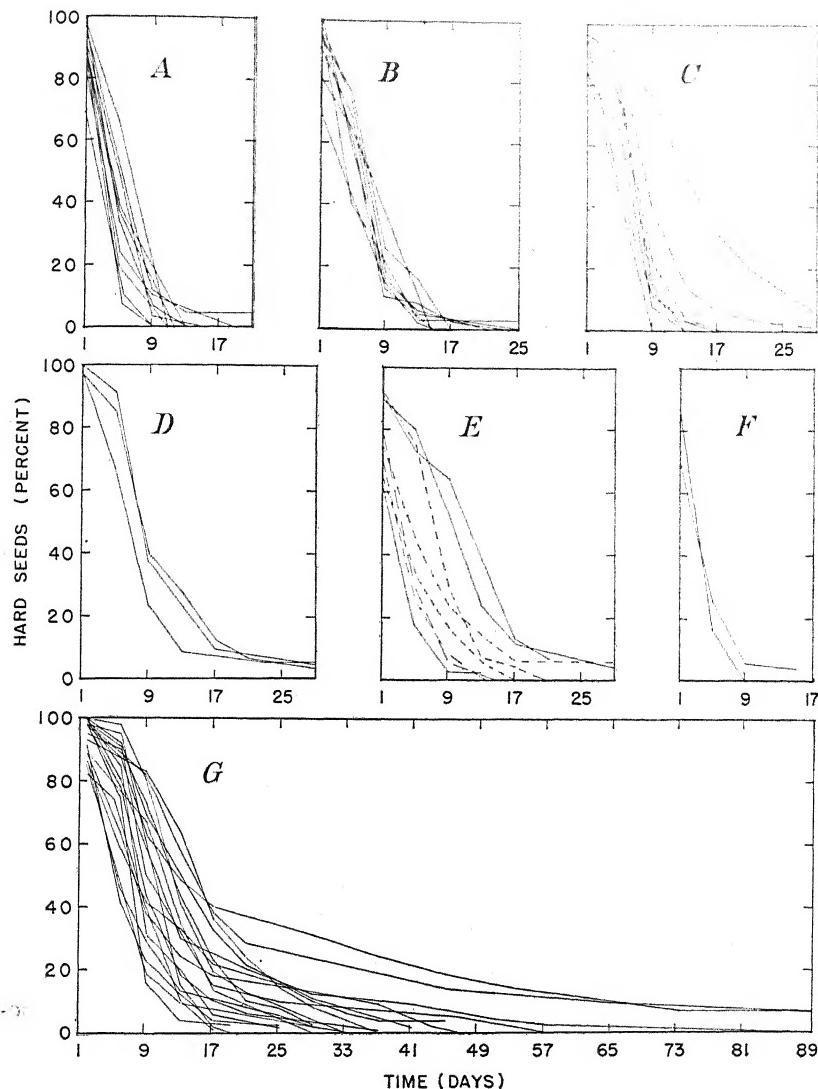


FIGURE 1.—Percentage of hard seeds on successive days of tests. A, selection 1099; B, selection 368; C,  $F_1$  from  $1083 \times 1099$  (solid lines) and reciprocal crosses (broken lines); D,  $F_1$  from  $475 \times 1099$  crosses; E,  $F_1$  from  $1083 \times 368$  (solid lines) and reciprocal crosses (broken lines); F,  $F_1$  from  $1099 \times 368$  crosses; G,  $F_2$  from  $475 \times 1099$  crosses. The  $F_1$  and  $F_2$  seeds from various crosses in figures 1 to 5 refer to the seed produced by the  $F_1$  and  $F_2$  plants respectively.

All germination tests were made in a Minnesota electrically heated germinator, model No. 3, in which the air temperature was approximately  $28^\circ$  C. The remaining 800 lots of seed were removed from the cheesecloth bags and placed in the germinator on the wire mesh trays which were covered with two thicknesses of cotton flannel cloth. At the beginning of the test, counts of hard and swollen seeds were made

every other day, and the swollen seeds were removed. After 35 days counts were made every 4 to 8 days.

Out of six selections which were intercrossed, only the results of crosses involving five selections, Nos. 1099, 368, 1083, 475, and 1130, will be described.

#### HARD SEEDS IN PURE LINES

Under the new environment and with the different methods employed in this experiment for drying and germinating of the seeds, hard-seed production was somewhat modified in several selections from that reported previously.<sup>5</sup> It is suspected that the quick drying at a high temperature was mainly responsible for this.

The 2 "soft-shelled" selections, 1099 and 368, were practically unchanged and the hard-seed production in them closely resembled that previously reported. Although the proportion of hard seeds was high in the seed of these selections, the hard seeds softened in a very short time. More than 20 plants of the 1099 selection were available, and seeds from most of them became soft by the thirteenth day of the test (fig. 1, A). In the 20 plants of selection 368 most of the seed became soft by the seventeenth day of the test (fig. 1, B). While there was a certain amount of variability in the hard-seed production from individual plants of both selections, the differences were slight.

All three "hard-shelled" selections, namely, 475, 1083, and 1130, became considerably harder in this experiment than they had in the earlier studies, and the time required for the softening of hard seeds of these selections was considerably longer.

Selection 475 was most drastically changed, and the usual drop in the number of hard seeds expected during the first few days of the germination test did not occur at all. Instead, the number of hard seeds in this selection decreased very slowly, and even after more than 200 days a large percentage of seeds remained hard (fig. 2, B).

In selection 1083 the softening of seeds was prolonged also but not to the same extent as in selection 475. In the earlier experiments conducted in Puerto Rico most of the seed of this selection at approximately the same moisture content had softened by the twenty-fifth day of the germination test, whereas, in the present experiment, softening of seeds of some plants of this selection was not complete even after 80 days. There was, however, considerable variation in the production of hard seeds in the individual plants in selection 1083 (fig. 4, D).

Selection 1130 appeared to be least changed, and the graphs of seed softening almost parallel those previously reported (fig. 3, A).

#### INHERITANCE OF HARD-SEED SOFTENING IN CROSSES

##### CROSSES INVOLVING SELECTIONS 1099 AND 368

The rate of seed softening in selections 1099 and 368 was essentially the same. The proportion of hard seeds in these selections usually was large, yet the hard seeds softened very quickly. Despite their similar behavior, these two selections were found to be different genetically in regard to hard-seed production. In crosses with 475, selection 1099 was found to be nearly completely dominant, whereas selection 368 was nearly recessive.

<sup>5</sup> See footnote 2, p. 205.

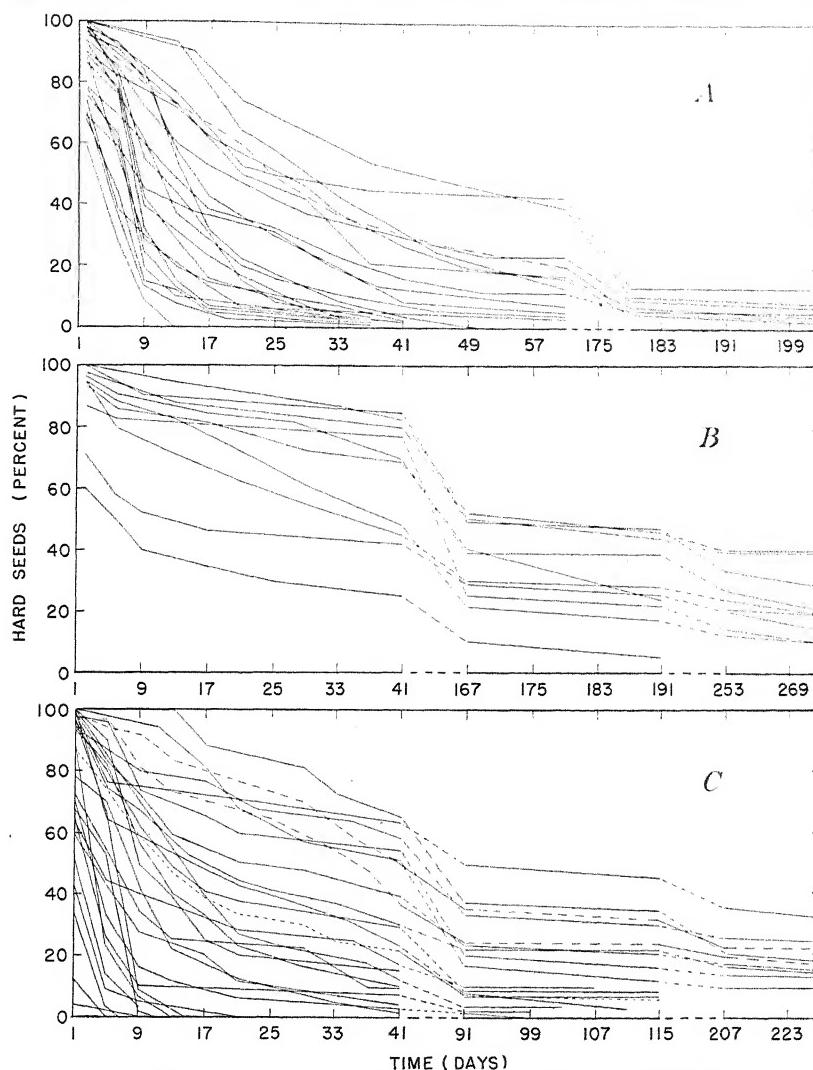


FIGURE 2.—Percentage of hard seeds on successive days of tests. *A*,  $F_1$  (broken line) and  $F_2$  (solid lines) from  $368 \times 475$  crosses; *B*, selection 475; *C*,  $F_1$  (broken lines) and  $F_2$  (solid lines) from  $1083 \times 1130$  crosses.

In the  $F_1$  from the  $475 \times 1099$  cross only 3 plants were available (fig. 1, *D*), and the seeds from these plants softened almost as rapidly as those of the 1099 parent. Twenty-one  $F_2$  plants were available from the  $475 \times 1099$  cross (fig. 1, *G*). In this population possibly 1 or 2 plants could be classified as producing hard seeds similar to those of the 1099 parent. In the rest of the plants the seeds softened considerably more slowly than those of the 1099 parent, but they were not nearly as slow in softening as those of the 475 parental selection.

In the 1  $F_1$  plant available from the  $368 \times 475$  cross the seed softening was nearly as slow as that of seeds of the 475 parent (fig. 2, *A* and *B*).

Of the 22 plants in the  $F_2$  from the  $368 \times 475$  cross, 1 could be classified as producing seeds which softened readily like those of the 368 parent, and 1 or 2 which produced seeds that softened at the very slow rate of the 475 parent. In the remaining plants the seed softening ranged between these two extremes. Eighteen  $F_2$  plants available from the reciprocal ( $475 \times 368$ ) cross gave essentially similar results.

Although selections 1099 and 368 proved to be genetically unlike when crossed with selection 475, they gave practically the same results in crosses with 1083.

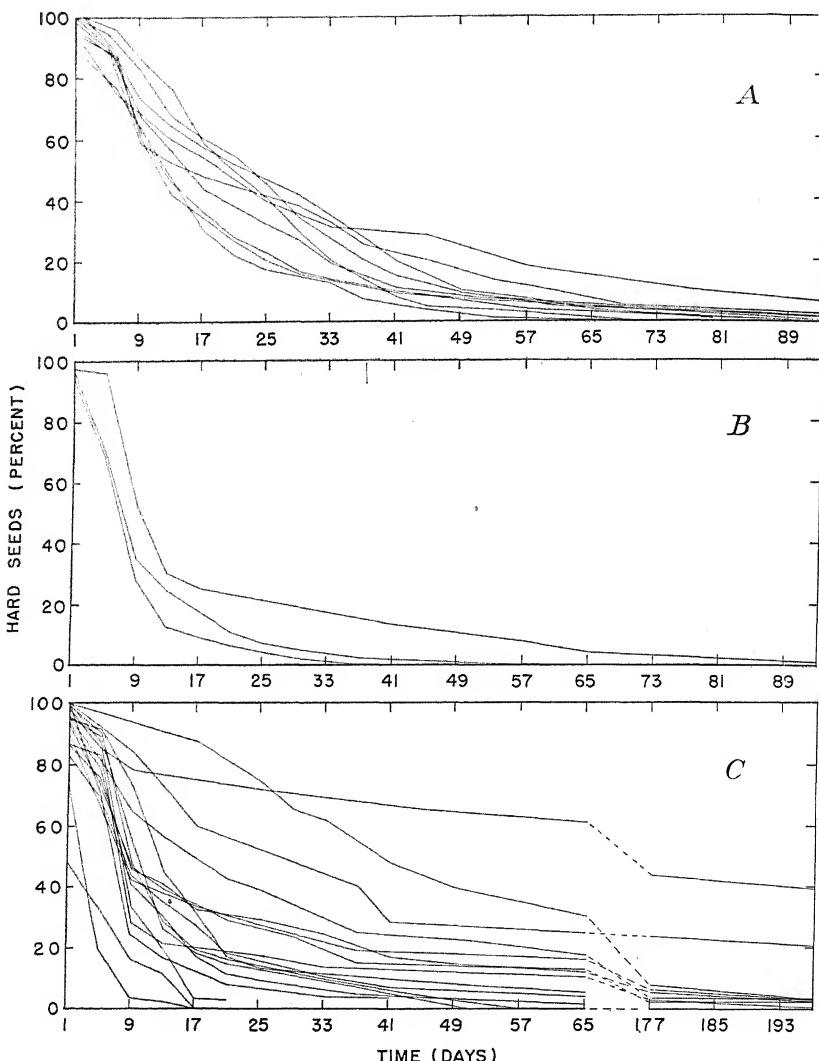


FIGURE 3.—Percentage of hard seeds on successive days of tests. A, selection 1130; B, F<sub>1</sub> from  $368 \times 1130$  crosses; C, F<sub>2</sub> from  $368 \times 1130$  crosses.

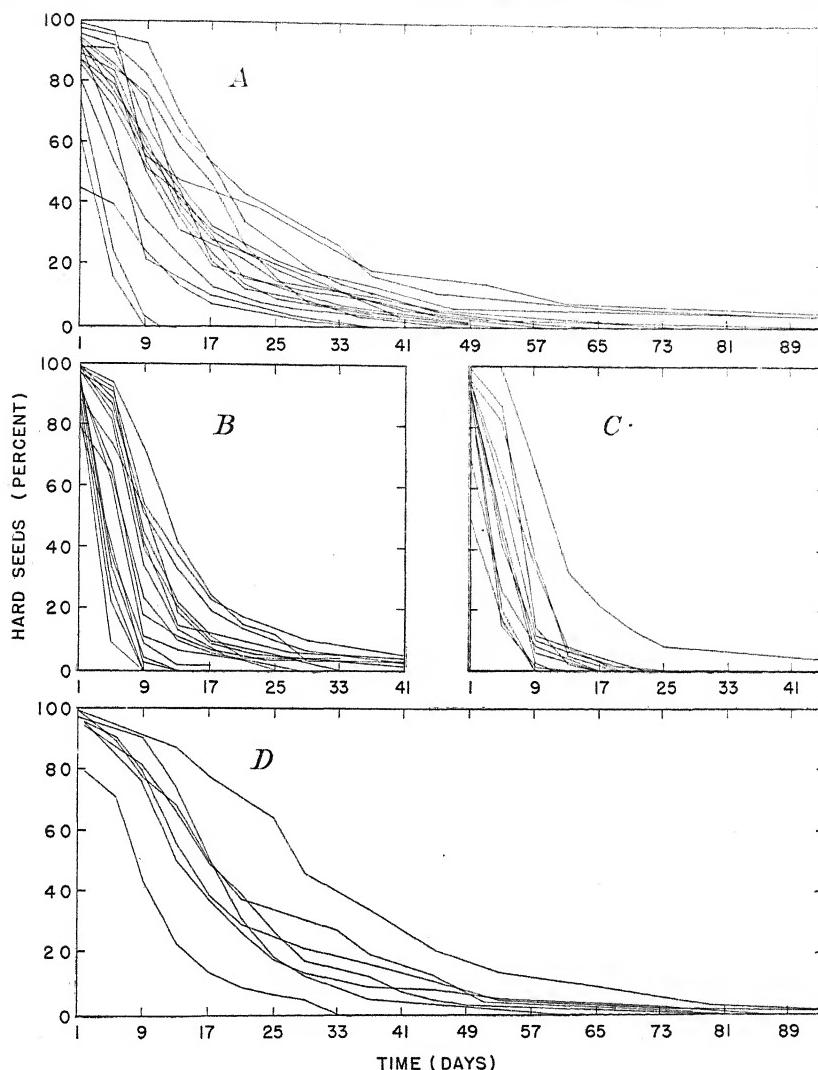


FIGURE 4.—Percentage of hard seeds on successive days of tests. *A*,  $F_2$  from  $1083 \times 1099$  crosses; *B*,  $F_2$  from  $1099 \times 1083$  crosses; *C*,  $F_2$  from  $1099 \times 368$  crosses; *D*, selection 1083.

There were 8  $F_1$  plants available from the  $1099 \times 1083$  and reciprocal crosses (fig. 1, *C*). In 7 plants seed softening was very rapid, similar to or approaching that of the 1099 parent, while in 1 plant it was slower and approached that of the 1083 parent. In 17  $F_2$  plants from the  $1083 \times 1099$  cross, seed softening varied from the very fast, as in 1099 to that of the slower softening 1083 parent, with the majority of plants showing seed softening of variously intermediate rates (fig. 4, *A*). In the  $F_2$  from the reciprocal cross ( $1099 \times 1083$ ) essentially sim-

ilar segregation occurred but with less dispersion and with the graphs of seed softening inclining toward that of the 1099 parent (fig. 4, *B*).

There were 8  $F_1$  plants available from the  $368 \times 1083$  and reciprocal crosses (fig. 1, *E*), and 22  $F_2$  plants from the  $1083 \times 368$  crosses (fig. 5, *A*). The seed softening in both  $F_1$  and  $F_2$  plants from these crosses was very similar to that found in crosses of selections 1099 and 1083.

The cross between the two "soft-shelled" selections 1099 and 368 was not well represented. In the  $F_1$  from the  $1099 \times 368$  cross, seeds of only 2 plants were available, and seed softening in them was like that of the 1099 parent (fig. 1, *F*). Seeds of 13  $F_2$  plants were available, and seed softening varied between that of the 2 fast softening parental selections or slightly exceeded that of the 368 parent (fig. 4, *C*).

Summarizing the results of crosses involving selections 1099, 368, 1083, and 475, it may be pointed out that seed softening in the  $F_1$  plants, under the conditions of this experiment, either closely approached that of the "softer" parent or was intermediate between that of the two parental selections. Seed softening of the  $F_2$  progenies usually ranged from that found in the "soft-shelled" parent to that found in the "hard-shelled" parent, with the majority of plans exhibiting all possible degrees of variation between the two extreme parental types.

#### CROSSES INVOLVING SELECTION 1130

Somewhat different results were obtained in crosses involving selection 1130.

There were three  $F_1$  plants available from  $1083 \times 1130$  crosses (fig. 2, *C*). The softening of the seed from these plants was considerably slower than that of either parental selection, as may be seen from the duration of the germination test. In the  $F_2$  there were 26 plants available from the  $1083 \times 1130$  cross (fig. 2, *C*), as well as 22 plants from the reciprocal cross. In both populations the rate of seed softening varied from very fast, even faster than that of seed of "soft-shelled" selections 1099 and 368, to very slow, even slower than that of either parent or than that of the  $F_1$  plants from crosses between them.

Seed softening in 9  $F_1$  plants from the  $1130 \times 475$  and reciprocal crosses was very slow and closely resembled that of the 475 parent (fig. 5, *B*). Thirty-one  $F_2$  plants from the  $1130 \times 475$  cross (fig. 5, *C*), as well as 34  $F_2$  plants from the reciprocal crosses were available. In both populations, the seeds ranged from a very fast softening type to a very slow softening type, with possibly a few individuals softening even more slowly than the 475 parent.

The results of the cross of 1130 with the soft-shelled selection 368 is somewhat different from those found in the crosses described above. In the three  $F_1$  plants from the  $368 \times 1130$  cross seed softening was intermediate between that of the two parental selections (fig. 3, *B*). For the first 15 days of the germination test the slope of the seed-softening curves for these  $F_1$  plants resembled that of the 368 parent, but after 15 days it approached that of the 1130 parent. There were only 20  $F_2$  plants available from the  $368 \times 1130$  cross (fig. 3, *C*). Seed softening in these plants ranged from that of the fast softening 368 parent to very slow softening, even slower than the 1130 parent. It is to be noted that in this  $F_2$  population the extremely soft types, such as were found in the crosses of 1130 with 1083 and 475, were not observed.

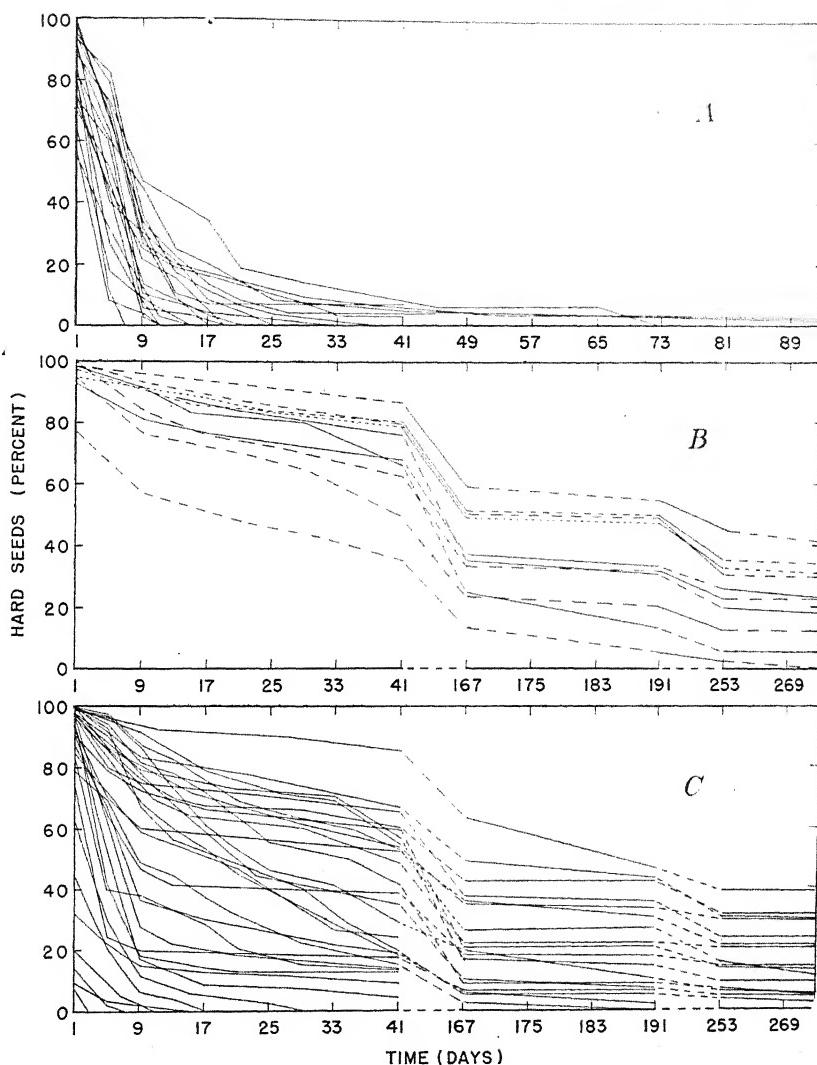


FIGURE 5.—Percentage of hard seeds on successive days of tests. *A*,  $F_2$  from 1083  $\times$  368 crosses; *B*,  $F_2$  from 475  $\times$  1130 (solid lines) and reciprocal (broken lines) crosses; *C*,  $F_2$  from 1130  $\times$  475 crosses.

#### DISCUSSION

Woodworth<sup>6</sup> made a cross between a soft-shelled and a hard-shelled variety of soybean, and soaked 25 of the  $F_1$  seeds and 10 seeds from each of the  $F_2$  plants in water for 8 days. He found soft-seededness (ability to take up water rapidly) to be partially dominant over hard-

<sup>6</sup> WOODWORTH, C. M. GENETICS OF THE SOYBEAN. Amer. Soc. Agron. Jour. 25: 36-51, Illus. 1933.

seededness. In the  $F_2$  the seed softening varied from very fast, even faster than that of seed of the soft-shelled variety, to very slow, even slower than that of the hard-shelled parent.

In previous studies<sup>7</sup> the writer found that there were inherent differences among pure lines of beans, both in the amount of so-called hard seed produced under a given set of environmental conditions, and in the rates of softening of the seed coat in these impermeable seeds. By selecting several pure lines which exhibit marked differences in rate of seed softening and intercrossing them, it was possible to study the inheritance of this character which is fundamentally a physiological one.

As in other studies of the inheritance of developmental processes and quantitative characters,<sup>8</sup> the inheritance of seed softening does not exhibit a clear-cut segregation in the  $F_2$  population but is rather a continuous variation from a very fast to a very slow rate of softening, the extremes often exceeding those found in the parental selections. No attempt will be made to evaluate the number of genes involved in the inheritance of seed softening as the segregating populations were too small to permit definite conclusions. The fact that the parental types of seed softening often were recovered in small  $F_2$  populations does indicate that only a few genes were involved in a given cross.

The effect of the environment on hard-seed production must not be overlooked, for the results described in this study could be duplicated only under similar conditions. Changing either environment or methods of drying would probably give different results. As the writer has previously shown, there are probably two independent physiological factors involved in hard-seed production, namely, the development of a certain proportion of hard seed and the differential rates of softening of these impermeable seeds.

Under the conditions of the present experiment the proportion of hard seeds was high in all six selections, and, therefore, only the inheritance of the rate of softening could be studied. Under a different environment the proportion of hard seeds would be high in some selections and relatively low in others, and it would be possible to study not only the rate of softening but also the range of occurrence of hard seeds. Under still another environment, neither of these processes could be studied. There were small differences in the number of hard seed produced and the rate of softening of impermeable seeds even between such contrasting selections as 1099 and 475, when their seed had a relatively high moisture content. Under such conditions the segregating populations from such a cross are very similar. In the light of these facts it appears that hard-seed production in beans or other legumes offers an exceedingly favorable means of studying the inheritance of physiological reactions in plants.

It is evident that breeding offers the best approach to the problem of eliminating hard-seededness. In a previous study and in the present report, it has been shown conclusively that hard-seededness in beans is heritable. Although there is no detailed factorial analysis, the writer's study indicates that only a few genes are involved in the

<sup>7</sup> See footnote 2, p. 205.

<sup>8</sup> SMITH, H. H. RECENT STUDIES ON INHERITANCE OF QUANTITATIVE CHARACTERS IN PLANTS. *Bot. Rev.* 10: 349-382. 1944.

differentiation of hard and soft-seeded selections. Therefore, in a breeding program with legumes an effort should be made to avoid using hard-seeded genotypes, particularly the slow-softening types. If soft-seeded strains are not available, they can be obtained from crosses between hard-seeded ones.

#### SUMMARY

Five selections of beans which exhibited marked differences under certain environmental conditions in regard to the rate of softening of hard seeds were intercrossed in various ways.

The plants of the original selections, as well as  $F_1$  and  $F_2$  plants from crosses between these selections, were grown together in a single environment.

After harvesting, seeds from these plants were first stored in the laboratory and then were kept for 5 days in an electric drier, in which the moisture content of the seed was reduced to about 6.61 percent.

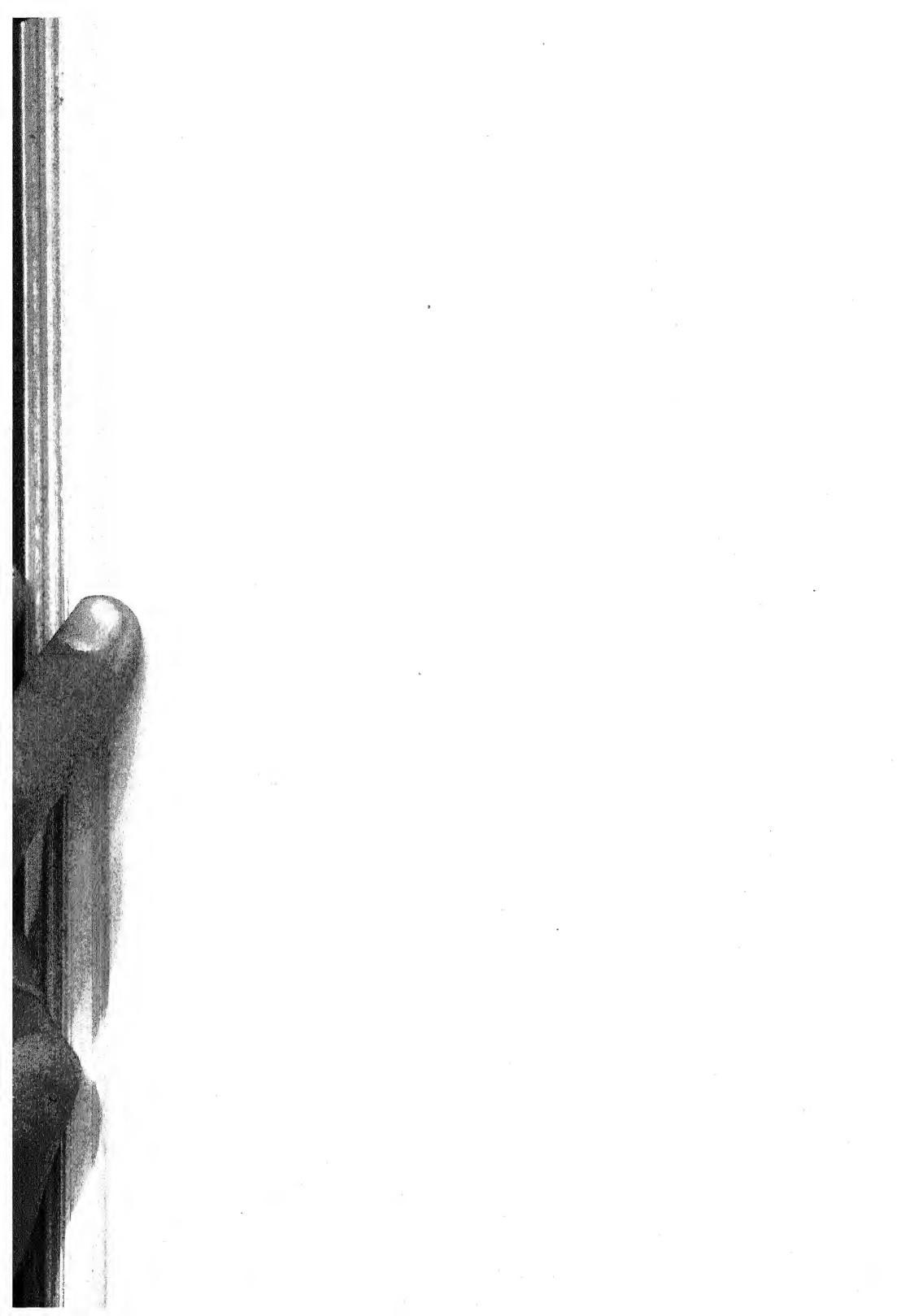
Upon removal from the drier, the seed was placed in a germinator and records were kept of hard and swollen seeds throughout the test. Under these conditions the two "soft-shelled" selections were unchanged and seed softening was like that reported in previous studies, while in three "hard-shelled" selections seed softening was even more prolonged.

Seed softening in various  $F_1$  crosses either approached closely that of the fast-softening parent or was intermediate between that of the two parental selections.

In the  $F_2$  seed softening ranged between that found in the two contrasting parental selections, often with practically all possible degrees of variation between these two extremes represented.

Seed of the  $F_1$  crosses involving selection 1130 were extremely slow in softening. The  $F_2$  seed exceeded in both rapidity and slowness the rates of softening in the two contrasting parental types; that is, some softened faster than the fast-softening parent, and others softened more slowly than the slow-softening parent, the majority softening at variously intermediate rates.

In the progenies other than those of the 1130 crosses, the parental extremes were not exceeded but were usually recovered. Since  $F_2$  populations were small only a few genes appear to be involved.



# THE RELATION BETWEEN AGE, NUMBER, AND TYPES OF CELLS IN THE PERIPHERAL CIRCULATION OF CHICKEN EMBRYOS UNDER NORMAL AND EXPERI- MENTAL CONDITIONS<sup>1</sup>

By R. A. FENNELL<sup>2</sup>

*Associate Professor of Zoology, Michigan Agricultural Experiment Station*

## INTRODUCTION

An extensive review and summary of the literature on natural transmission of the avian leukosis complex in the fowl has been made by Jungheer (13).<sup>3</sup> At one time or another three routes of transmission have been considered by workers in this field; namely, (1) through the egg, (2) by contact, and (3) by mechanical vectors such as insects. The review shows, however, that the mode of transmission of this disease is still open to question.

Waters and Prickett (22) and Waters (21) report that chickens hatched from eggs of infected dams, reared in complete isolation under rigid quarantine, showed an incidence of lymphomatosis of 16 percent, while other progeny from disease-free dams, reared in isolation and likewise quarantined, showed no lymphomatosis. On the basis of these observations, these authors suggest that lymphomatosis may be an egg-borne disease.

If the disease is egg-borne, quantitative, or qualitative cytological differences of such magnitude may be present in the peripheral circulation of embryos from lines of chickens which show various degrees of resistance and susceptibility to lymphomatosis as to make positive identification of the disease possible. It is well known that pronounced cytological variations are found occasionally in the peripheral blood of adult chickens infected with lymphomatosis (1, 10, 13).

The work on the developmental and genealogical history of the various cell types in the hemopoietic foci of the chicken embryo has been ably reviewed by several authors (5, 7, 9, 20). However,

<sup>1</sup> Received for publication April 12, 1946. Journal article No. 806 (n. s.) of the Michigan Agricultural Experiment Station.

<sup>2</sup> The writer is indebted to Berley Winton, director, and to staff members of the U. S. Regional Poultry Research Laboratory for a grant-in-aid and for the use of laboratory facilities; to Dr. N. F. Waters, geneticist, for making available the hatching eggs from inbred chickens resistant and susceptible to lymphomatosis; and to Dr. B. R. Burmester, physiologist, for supplying the RPL-17 tumor mince; and to Dr. C. O. Prickett, pathologist, for taking the photomicrographs shown in figure 2.

<sup>3</sup> Italic numbers in parentheses refer to Literature Cited, p. 238.

only two reports, those of Sugiyama (20) and Dawson (8), have been primarily concerned with the relative numbers of the various cell types in the peripheral circulation of chicken embryos. Dawson based his conclusions on the study of only a limited number of specimens. Furthermore, he used the terminology of the unitarian hematologists while Sugiyama used that of the so-called dualistic hematologists. For this reason it was difficult to correlate the results given in the two papers. It therefore seemed advisable to make a differential count of the various cell types in the peripheral circulation of the chicken embryo to ascertain the "normal" frequency of occurrence of the cell types in the peripheral circulation in embryos of various ages.

The objectives of the present study were twofold: (1) To identify and ascertain the mean percentages of the various cell types in the peripheral circulation of inbred embryos of various ages from lines of chickens which showed various degrees of resistance and susceptibility to lymphomatosis; and (2) to study the effects produced on the peripheral blood of embryos by the injection of both normal and pathological tissues.

#### MATERIALS AND METHODS

The embryos used in this study were from lines of Single-Comb White Leghorn stock which showed various degrees of resistance to lymphomatosis (42). All embryos carry the number of the dam.

The majority of the blood samples used in this study were obtained from the heart with a micropipette. Two hundred or more embryos were studied by the supravital methods of Cunningham and Tompkins (3). An additional 8 to 20 duplicate samples of blood were obtained for each day of incubation from 48 hours until the day of hatching, and then they were stained with Wright and May-Grünwald Giemsa stains. All blood samples were examined, and preliminary differential counts of the cells of several fields in each of the blood smears were made to ascertain the extent of variation for each age. Blood samples which seemed to show all cytological variations for a given age were then selected for differential counts. From 4,000 to 11,000 cells were counted for each age group. Counts were made at 72-hour intervals. The mean diameters of cells and nuclei were ascertained by making 25 measurements with a Zeiss screw eyepiece micrometer.

#### OBSERVATIONS

##### EMBRYOS OF 71-76 HOURS' INCUBATION

###### VARIATIONS IN CELL TYPES

Table 1 and figures 1 and 2 show that two generations of red blood cells, the primitive and definitive, are present in the peripheral circulation of the chicken embryo. The photomicrographs in figure 2, A, B, and C, reveal that the peripheral circulation of embryos of 71 to 76 hours' incubation contain primitive erythroblasts and hemocytoblasts. In embryos F1009 and F1043, the large primitive erythroblasts with medium hemoglobin had a mean diameter of  $12.91\mu$  and  $11.39\mu$ , respectively. The nucleus in the former had a diameter of  $7.28\mu$ , while that of the latter was  $8.03\mu$  (Wright stain). In vital

preparations the cytoplasm of these cells (fig. 1, G) had a yellowish tinge, and in most preparations a rosette of neutral red bodies could be identified in the cytoplasm midway between the nucleus and cell wall. Figure 1 shows that small particles of chromatin were present in the nucleus. Preparations that were left on a warm stage at 34.5° for 4 to 6 hours frequently contained some erythroblasts with blebs on the surface; these blebs in many instances pulled away from the cell and became free. The primitive erythroblast has been recognized by many writers as the first hemoglobin-containing cell of the vertebrate embryo.

TABLE 1.—*Mean percentage of cells of various types in the peripheral blood of embryos of various ages*

Embryo number	Age	Cells counted	Primitive erythroblasts		Primitive erythrocytes		Definitive erythroblasts		Definitive erythrocytes		Degenerating cells
			M e d i u m hemoglobin	M u c h hemoglo- bin	M e d i u m hemoglobin	L i t t l e hemoglo- bin	M e d i u m hemoglobin	Hemocytoblasts	Thrombocytes		
			Number	Percent	Percent	Percent	Percent	Percent	Percent	Percent	
F1009 F1029	71-76	3,310	96.64	0	0	0	0	0	1.74	0	1.27
F1029 F1040											
F1043, F1050											
F1051											
E453, E608											
E680, E701	93-100	4,100	95.50	0	0	0	0	0	1.96	0	2.51
F1051, F1056, F1460-2											
E190, E213, E347, E348, E635, E691, E799	139-148	5,172	0	66.11	.40	4.85	12.43	10.61	.58	0	4.91
F1043											
E190, E321, E440, E605, E609, E797, ES14, E882, 50, E894-1, E888-1, E894- 3, E888-2, E894-3, (2) E888-3, E894-5	213-217	6,300	0	5.14	1.85	.14	1.74	90.22	.03	0	.85
E888-2, E893-2, E903-1, E894-6, E907-1, E897- 3, E907-2	283-288	6,200	0	6.59	0	.13	3.72	88.29	0	.09	1.01
E888-3, E894-5											
E888-2, E893-2, E903-1, E894-6, E907-1, E897- 3, E907-2	356-360	6,000	0	2.65	.03	.03	.91	95.38	0	.21	.55
E881-1, E888-3, E894-4, E1460-1, E1460-3	425-431	5,600	0	.09	0	0	0	99.16	0	.68	.03
E898-1, E905-4, E905-1, E907-4, E907-2	475-480	5,000	0	.06	0	0	.06	98.52	.02	1.24	0

In embryo F1009 primitive erythroblasts with medium hemoglobin had a mean diameter of  $9.84\mu$ , and in embryo F1043 (71-76 hours' incubation), the mean diameter was  $8.11\mu$ . These primitive erythroblasts appeared to be derived from the large erythroblasts since mitotic division reached 0.39 percent, and in the telophase of these divisions the diameter of the daughter cells approximated that of the medium-sized erythroblasts. In supravital preparations a portion of the large primitive erythroblasts appeared to divide atypically to form medium-sized erythroblasts.

Figure 2, A and B, shows hemocytoblasts of various sizes found in the peripheral circulation of embryos of 71-76 hours' incubation. The largest of the hemocytoblasts correspond to the lymphocytes of Dantschakoff (4, 5, 6, 7). This author believed that such lymphocytes were morphologically, as well as functionally, essentially the same as

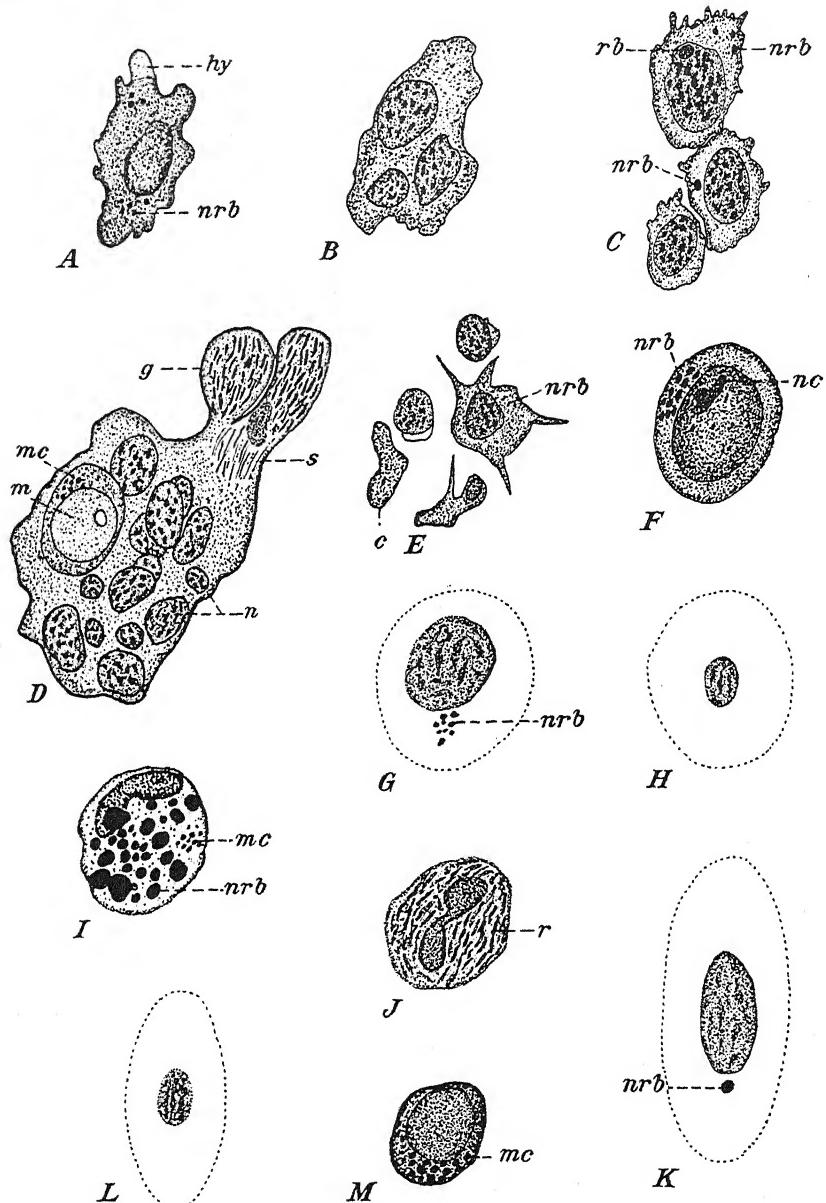


FIGURE 1.—*Camera lucida drawings of peripheral blood (supravitally stained with neutral red and Janus green) of chicken embryos of various ages: A, Hemocytoblast, 71-74 hours' incubation; B, multinucleated hemocytoblast, 93-96 hours' incubation; C, fragmentation of a hemocytoblast to produce thrombocytelike cells; D, giant cells, 257-260 hours' incubation, showing granulocyte differentiating from surface; E, fragmentation of giant cell to produce thrombocytelike cells, 285-288 hours' incubation; F, transitional hemocytoblast (primitive erythroblast), 117-120 hours' incubation; G, primitive erythro-*

the lymphocyte of the adult chicken. The smallest hemocytoblast identified seemed to agree morphologically with the dwarf lymphocyte of Dantschakoff.

The hemocytoblast in the peripheral circulation of embryos of 71–76 hours' incubation, exhibited in Wright-stained preparations a pronounced basophilic cytoplasm which formed blebs that were frequently separated from the cell (fig. 2, A), leaving the nucleus naked or almost so at the point of separation. Not all hemocytoblasts, however, showed this tendency to form blebs; in fact, bleb formation seemed to be an artifact since this phenomenon was not seen in fresh vital preparations. The nucleus was large, often reaching a mean diameter of  $8.79\mu$ . Frequently, the nucleus seemed to be empty, and when this was the case it exhibited a weak basophilia; at other times, the small dustlike chromatin particles were uniformly distributed, and gave to the nucleus a light, sky-blue, stippled appearance. One or more nucleoli were frequently seen and these were usually a darker, robin's-egg blue.

In vital preparations the cytoplasm of the hemocytoblast was colorless and had a ground-glass appearance (figs. 1, A and B). A variable number of neutral red bodies were sometimes observed in the cytoplasm. Pseudopod formation was rather common, and at the distal tips of the pseudopods a hyaline cap was evident in some specimens. One or more nucleoli were frequently found in the nucleus. A refractive body was sometimes seen in the cytoplasm in about the same position in the cell as the centrosome in stained preparations.

Table 1 shows that the mean percentage of degenerating cells in embryos incubated for 71–76 hours was 1.27. These cells occurred singly or in clumps of three, four, or more. The cytoplasm was either weakly basophilic, acidophilic, or neutrophilic and formed a narrow, irregular border around the nucleus. Some nuclei were naked, the chromatin within the nuclei had undergone a pronounced condensation, and its affinity for basic dyes had increased; i. e., the nuclei showed a pronounced pyknosis. In some blood smears the nuclei resembled those of small hemocytoblasts; in others they looked more like those of erythroblasts. In one clump of six cells, the largest nucleus in the group had a diameter of  $5.75\mu$  and the smallest,  $3.07\mu$ .

#### VARIATIONS IN NUMBER OF CELL TYPES IN INDIVIDUAL BLOOD SAMPLES

The number of primitive erythroblasts counted in each of the smears used for arriving at the mean percentage given in table 1 for embryos of 71–76 hours' incubation were in fairly close agreement. The varia-

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blasts with medium hemoglobin, 91–94 hours' incubation; *H*, primitive erythroblast (orthochromatic) 141–144 hours' incubation; *I*, myelocyte, 285–288 hours' incubation; *J*, polymorphonuclear heterophilic granulocyte, 284–287 hours' incubation; *K*, primitive erythrocyte, 141–144 hours' incubation; *L*, definitive erythrocyte, 141–144 hours' incubation; *M*, hemocytoblast 6 hours after hatching.  $\times \frac{1}{4}$ .

*c*, Cytoplasm; *g*, polymorphonuclear heterophilic granulocyte; *hy*, hyaline layer; *m*, hemocytoblast; *mc*, mitochondria; *n*, nuclei; *nc*, nucleolus; *nrb*, neutral red body; *r*, spindle-shaped crystalline bodies; *rb*, refractive body; *s*, folds. Bausch and Lomb, 2-mm. apochromatic objective with compensating ocular 10 was used. Drawings made at 1.8 cm. above table level.

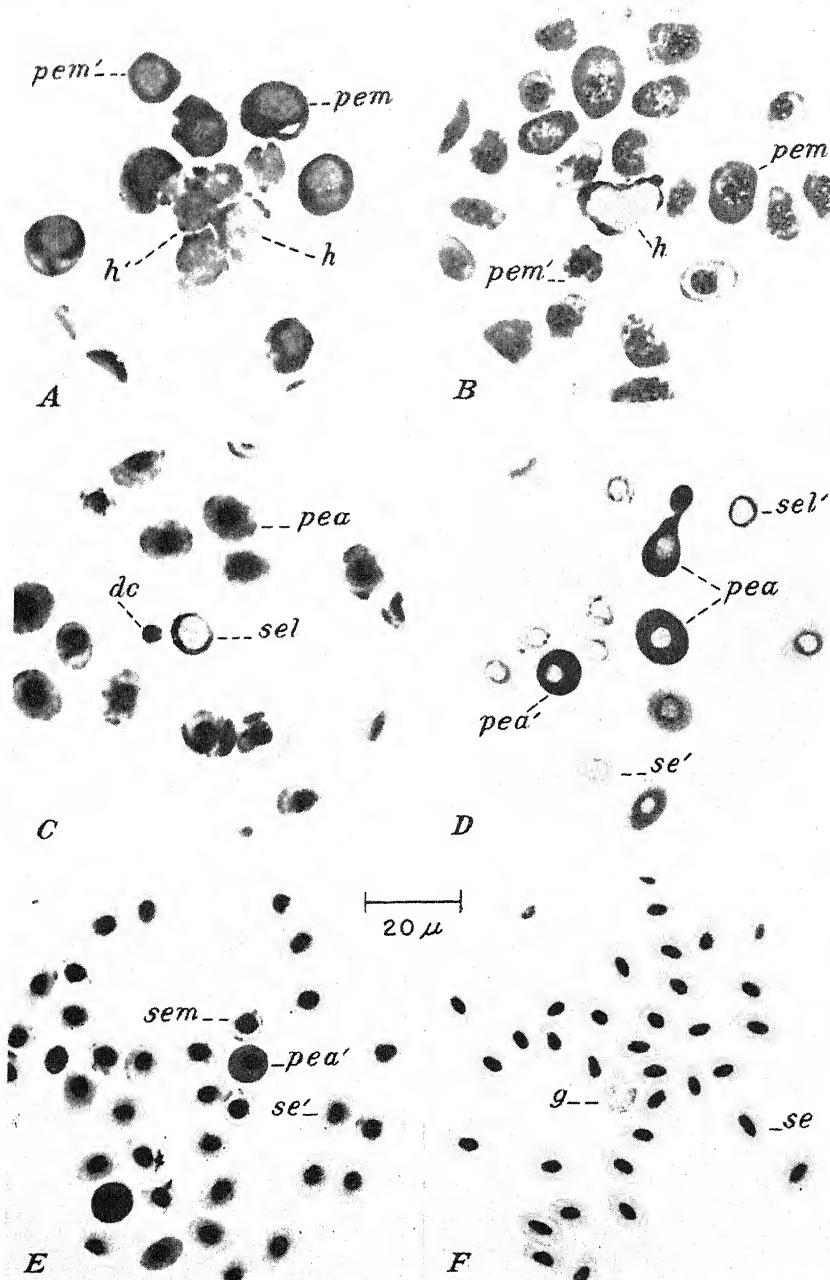


FIGURE 2.—Photomicrographs of peripheral blood (May-Grünwald Giemsa stain) of chicken embryos of various ages: *A*, 69–72 hours' incubation; *B*, 97–100 hours' incubation; *C*, 145–148 hours' incubation; *D*, 284–287 hours'

tions observed in number of hemocytoblasts (small, medium, and large) were more striking. In embryos from susceptible line 15, a maximum of 21 hemocytoblasts was found in F1050 (500 cells counted), and a minimum of 3 in F1029 (300 cells counted). In susceptible embryo F1040 from line 2, 11 hemocytoblasts were found among the 410 cells counted.

In blood smears from embryos F1050, F1029 (line 15), and F1040 (line 2) one large cell with a pronounced vacuolization of the cytoplasm was observed in each of the three slides. The morphological features of this cell agreed fairly closely with the description given by Murray (16) for wandering cells.

On the basis of the observations made in this study and also from the descriptions given in the literature, it seems fairly safe to conclude that the vacuolated cells found in the peripheral circulation were the histiocytes of other authors.

#### EMBRYOS OF 93-100 HOURS' INCUBATION

##### VARIATIONS IN CELL TYPES

The primitive erythroblasts decreased from a mean percentage of 96.64 in embryos of 71-76 hours' to 95.50 in embryos of 93-100 hours' incubation (table 1). Proliferation must have been restricted mainly to the largest primitive erythroblasts since percentage mitosis (93-100 hours) was found to be 0.62. The hemocytoblasts (lymphocytes) increased from a total mean percentage of 1.74 in embryos of 71-76 hours to 1.96 in embryos of 93-100 hours and, correspondingly, the degenerating cells increased from 1.27 to 2.51. Histiocytes were not identified in the latter and a further check was made by examining 150 fields in each slide prepared from embryos of 93-100 hours' incubation, but the results were negative.

##### VARIATIONS IN NUMBER OF CELL TYPES IN INDIVIDUAL BLOOD SAMPLES

All differential counts of cells in the embryos of 93 to 100 hours' incubation showed that the number of large erythroblasts was greater than the number of medium erythroblasts. Only 1 embryo (E701, line 12) from the inbred resistant lines was used for experimentation. This embryo showed only 1 hemocytoblast in a differential count of 500 cells, whereas the embryos from susceptible lines (4 from line 15, 2 from line 8) showed considerable variation in total number of hemocytoblasts. The highest number of hemocytoblasts (31 in 500 cells) was found in F1051, susceptible line 15, and the next highest number in F1460, embryo 2, susceptible line 15. The highest number of

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incubation; *E*, 356-359 hours' incubation; *F*, 429-431 hours' incubation.  
All  $\times 700$ .

*dc*, Degenerating cell; *g*, polymorphonuclear heterophilic granulocyte; *h*, large hemocytoblast; *h'*, medium-sized hemocytoblast; *pea*, large primitive orthochromatic erythroblast; *pea'*, medium-sized primitive orthochromatic erythroblast; *pem*, large primitive erythroblast with medium hemoglobin; *pem'*, medium-sized primitive erythroblast with medium hemoglobin; *se*, definitive erythrocyte; *se'*, immature definitive erythrocyte; *sel*, definitive erythroblast with little hemoglobin; *sel'*, small definitive erythroblast with little hemoglobin; *sem*, definitive erythroblast with medium hemoglobin.

degenerating cells (22) was found in E701, resistant line 12, and the lowest in E608 and E680, susceptible lines 15 and 8 respectively.

#### EMBRYOS OF 139-148 HOURS' INCUBATION

##### VARIATIONS IN CELL TYPES

The primitive erythroblasts with a medium amount of hemoglobin decreased to zero and the primitive erythroblasts with much hemoglobin (dark rose red in fixed preparations) increased from zero in embryos of 93-100 hours' incubation to a total mean percentage of about 66 in embryos of 139 to 148 hours' incubation (table 1). Dantschakoff (5) found that the primitive erythroblasts (orthochromatic) reached complete maturity on the fifth day of incubation and that at maturity the cytoplasm of the cells in fixed preparations (celloidin technique) stained pure red, the nucleus remained small, apparently shriveled, the nucleus became less clear, and mitotic activity ceased. Measurements made in this study showed that the large primitive erythroblasts with much hemoglobin had a mean cell diameter of  $13.16\mu$  and that the diameter of the nucleus was  $4.69\mu$ . Primitive erythroblasts of medium size with much hemoglobin had a cell diameter of  $10.19\mu$  and a nuclear diameter of  $3.89\mu$ , while the diameters of the cell and nucleus decreased to  $8.73\mu$  and  $3.66\mu$ , respectively, in the smallest erythroblast of this type.

Table 1 shows that the mean percentage of definitive erythroblasts with little hemoglobin was 4.85. In embryos E348 and E635, the diameters of the cell were, respectively,  $9.55\mu$  and  $9.13\mu$ , and those of the nucleus  $7.18\mu$  and  $7.00\mu$ .

Figure 1, *D*, shows a supravital preparation of the youngest cell in the definitive erythroblast series. This cell (*m*) was connected with the giant cells by a protoplasmic bridge. A comparison of this cell with the lymphocyte of the adult chicken (figure 1, *M*) shows the two to be similar in that the cytoplasm of both had a ground-glass appearance with oval or round mitochondria and limited amounts of chromatin in the nuclei. Wright-stained definitive erythroblasts with little hemoglobin likewise resembled lymphocytes (hemocytoblasts) of adult blood.

Table 1 shows that the definitive erythroblasts with medium hemoglobin (polychromatic) increased from zero in embryos of 93-100 hours to a mean percentage of  $12.43\mu$  embryos of 139-148 hours' incubation. The mean diameters of this cell (embryo E635) and its nucleus were  $8.22\mu$  and  $4.68\mu$ , respectively. It was evident in all slides that this cell was derived from the definitive erythroblasts with little hemoglobin, since transitional stages could be arranged in an orderly sequence from immature erythroblasts to more mature erythroblasts.

##### VARIATIONS IN NUMBER OF CELL TYPES IN INDIVIDUAL BLOOD SAMPLES

An analysis of individual counts of cells found in the peripheral circulation of the embryos incubated for 139 to 148 hours showed that the numbers of primitive and definitive erythroblasts used for obtaining the mean percentages in table 1 were approximately the same in resistant embryos E213 (line 6), E348 (line 6), and E635 (line 10), and that in resistant embryo E799 (line 13) the definitive erythroblasts

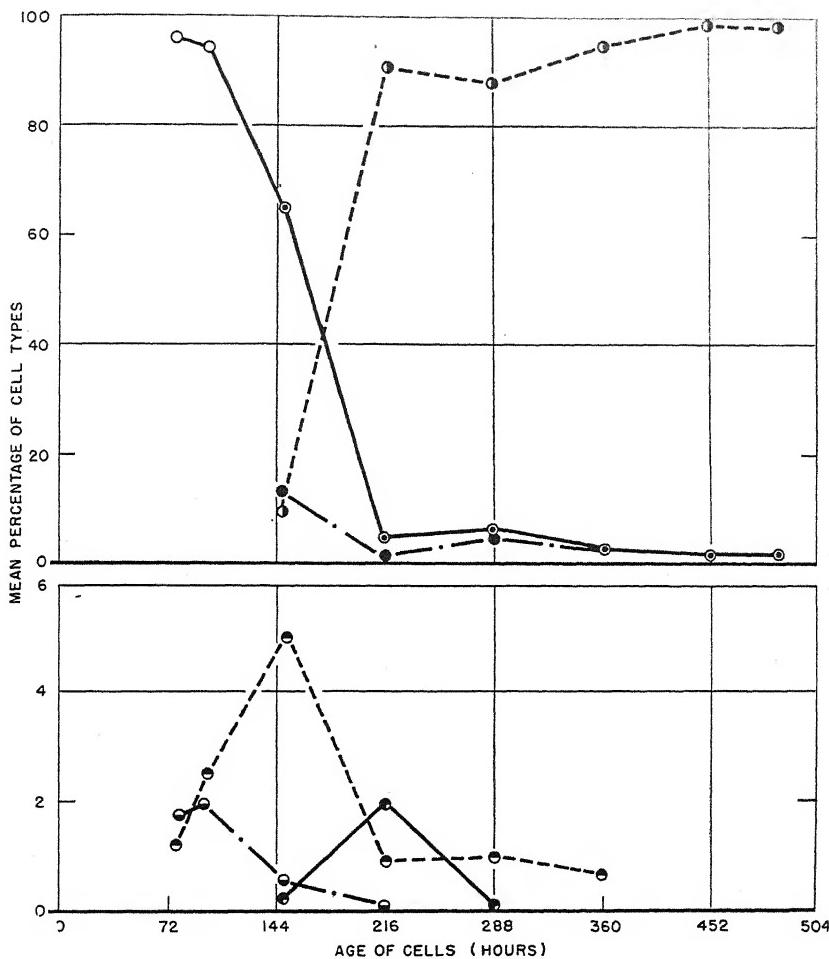


FIGURE 3.—Relation between age and mean percentage of cell types in the peripheral circulation of chicken embryos of various ages.

- = Primitive erythroblasts with medium homoglobin (polychromatic);
- = primitive erythroblasts with much hemoglobin (orthochromatic);
- ◐ = definitive erythrocytes;
- = definitive erythroblasts with medium homoglobin (polychromatic);
- ◐ = degenerating cells;
- ◑ = hemocytoblasts; and
- = primitive erythrocytes.

with little hemoglobin decreased to zero, while the definitive erythrocytes increased to 70 percent; consequently, the mean percentage for definitive erythrocytes given in table 1 is 10.61. Other striking variations in numbers of cell types were found in this resistant group of embryos. One of the highest numbers (687 in a differential count of 846) and also the lowest number (101 in 700) of primitive erythroblasts with much hemoglobin were found in resistant embryos E213 and E799, respectively. The number of hemocytoblasts remained fairly constant; a high of 5 cells in 797 cells was obtained for E635 and a low of 1 in 846 cells for E213. Four myelocytes were observed in E348 (409 cells counted). The smallest number of degenerating cells, 8 and 9, were found in E635 and E799, respectively. Four histiocytes were identified in 2 of 8 slides; 2 each in resistant embryo E190 and susceptible embryo E691.

A comparison of cell types in the peripheral circulation of individual susceptible embryos showed that the primitive and definitive erythroblasts were fairly constant in number, and a further comparison of susceptible with resistant embryos showed the numbers of primitive and definitive erythroblasts in the two groups to be in essential agreement. Other variations were noted, however, in the susceptible embryos. Ten hemocytoblasts (626 cells counted) were identified in embryo E347 (line 7), 2 (556 cells counted) in F1043 (line 7), and 6 (597 cells) in E691 (line 15). Degenerating cells were relatively abundant. Fifty-five degenerating cells in a total of 597 were found in embryo E691, 54 in 556 in F1043, and 50 in 626 in E347.

#### EMBRYOS OF 213-217 HOURS' INCUBATION

##### VARIATIONS IN CELL TYPES

The mean percentage of primitive erythroblasts with much hemoglobin decreased from about 66 in embryos of 139 to 148 hours' incubation to about 5 in embryos of 213 to 217 hours' incubation and the primitive erythrocytes increased from a mean percentage of 0.4 to about 1.85 (table 1 and fig. 3). The mean dimensions of the primitive erythrocyte in embryo E797 were  $12.74\mu$  long and  $8.95\mu$  wide with a nuclear diameter of  $4.18\mu$ . In Wright stain the primitive erythrocytes become a dark rose-red color. Dantschakoff (5) states that the primitive erythrocytes cannot be sharply separated from the primitive erythroblasts. She believes that degeneration of this so-called provisional red cell begins on the fourth or fifth day and is characterized by the nucleus becoming smaller, more shrunken, and paler.

The definitive erythroblasts with little hemoglobin decreased from a mean percentage of 4.85 in embryos of 139-148 hours to 0.14 in embryos of 213-217 hours, and there was a corresponding decrease in definitive erythroblasts with medium hemoglobin from 12.43 to 1.74. The definitive erythrocytes, however, increased from a mean percentage of 10.61 to 90.22. The definitive erythrocytes were smaller than the primitive erythrocytes and the affinity of their cytoplasm for eosin stain was less pronounced. The mean length of the definitive erythrocytes was  $10.32\mu$ ; width,  $7.00\mu$ ; diameter of nucleus,  $4.29\mu$ . Considerable variation was found in their staining reaction. Some stained grayish blue with a suggestion of pink; others were poly-

chromatic; and still others, the more mature ones, were light rose red. The affinity for eosin was never so pronounced in the definitive erythrocytes of either the embryo or adult as it was in the primitive erythrocytes. These differences in staining reaction and also in size were used as criteria for the identification of primitive and definitive erythrocytes. Hall (12) found that the affinity of hemoglobin for oxygen decreased in the chick embryo from the tenth day up to 3 weeks after hatching. Hemoglobin of early development, according to Hall, was replaced by a hemoglobin late in development which had less affinity for oxygen.

#### VARIATIONS IN NUMBER OF CELL TYPES IN INDIVIDUAL BLOOD SAMPLES

In the group of 213–217 hours' incubation, three embryos (E190, E440, E797) were from resistant line 13 and one (E882) from resistant line 10. A comparison of the data obtained by differential counts of cells in the peripheral blood of all resistant embryos showed that the frequency of occurrence of both erythroblasts (primitive and definitive) and hemocytoblasts was essentially the same in all the embryos. There was likewise little variation in the number of degenerating cells.

The embryos E321, E609, and E814 were from susceptible line 15, and E605 was from susceptible line 7. The differential counts showed that cell types in individual embryos of both lines 15 and 7 were fairly constant in number, and a comparison of the individual differential counts of the susceptible embryos with those of the resistant embryos showed no significant qualitative or quantitative differences.

#### EMBRYOS OF 283–288 HOURS' INCUBATION

##### VARIATIONS IN CELL TYPES

In embryos of 283–288 hours' incubation there were no primitive erythroblasts with medium hemoglobin, and the increase in primitive erythroblasts with much hemoglobin over the preceding age group was less than 2 percent (table 1).

The definitive series of red cells show similar variations in number. Erythroblasts with medium hemoglobin increased from a mean percentage of 1.74 to 3.72, and the definitive erythrocytes decreased from 90.22 to 88.29. These variations were not considered significant.

For the first time in this study thrombocytes appeared. Observations on vital preparations of peripheral blood of embryos of various ages revealed giant cells (fig. 1, D) with 15 or more nuclei. These may correspond to the clumps of hemocytoblastlike cells in Sugi-yama's stained preparations which were alleged to demonstrate thrombogenesis. Some of the nuclei in the giant cells, at least the smaller, more pyknotic ones, may be ingested red cells, since on numerous occasions red cells in vital preparations were observed in the process of being phagocytized. When the giant cells were maintained on a warm stage, bleblike structures frequently developed on the surface and sometimes these contained numerous cytoplasmic rod-shaped granules, with or without nuclei. In one vital preparation, rod-shaped granules were observed to collect in and around a kidney-shaped nucleus, and at this point there was no indication of a bleb; this was interpreted as an early stage in granulocyte forma-

tion. Observations made on 200 or more vital preparations lead the writer to believe that these giant multinucleated cells arose by fusion of stem cells which were identified by hemocytoblasts, and also by atypical nuclear division.

After the clumping of individual hemocytoblastlike cells, the cellular membranes seemed to disintegrate at the points of contact with other cells, and only that part of the membrane which was free and not in contact with other cells, was left intact. The resultant cells seemed to retain hemopoietic potentialities, and for this reason differentiation of granulocytes appeared to take place under the stimulus of unfavorable environmental factors (fig. 1, C).

Obviously blood samples, sealed with vaseline, on a warm stage are in an environment very different from that of the living animal. Under such conditions immature cells with fragile cell membranes probably disintegrate to produce extracts, and extracts may accelerate metabolic activity and facilitate differentiation of granulocytes. The writer (11) has found that extracts of paramecia facilitate attachment and ingestion in the large multinucleated rhizopod *Pelomyxa carolinensis*. The production of extracts in blood cultures was probably facilitated by the multinucleated cells themselves. Erythroblasts in contact with giant cells were frequently observed to lose their hemoglobin in the space of 1 or 2 seconds. Other erythroblasts, further removed, were normal in every respect. Thus it would appear that some material in the vicinity of the giant cell altered the cell membranes of erythroblasts and allowed the hemoglobin to escape.

The giant cells sometimes exhibited other developmental potentialities. If they were left at room temperature on the stage of a microscope, fragmentation frequently occurred. The resultant cells showed pyknotic nuclei with a ground-glass, metabolic cytoplasm, (fig. 1, C and E). Neutral red bodies were frequently found in the cytoplasm. Cells produced in this manner were similar morphologically to the thrombocytes. This cytological process in the giant cells was similar to fragmentation observed by the writer in the large multinucleated rhizopod *Pelomyxa carolinensis* when it was subjected to temperatures of 4° or 45°.<sup>4</sup>

Other observations made by the writer suggest that thrombocytes may be closely related to young erythroblasts. Stained preparations frequently showed round and elliptical thrombocytes with a stain-free, weakly acidophilic or basophilic, cytoplasm, and pyknotic nuclei. Eosin-staining granules were frequently found in the vicinity of the nucleus in the same relative positions as the neutral red bodies in supravitally stained preparations of erythroblasts and erythrocytes. Both in amount and pattern the chromatin in thrombocytes showed a pronounced similarity to that in young erythrocytes. Blount (1) believed that the thrombocyte was an erythrocyte in which the hemoglobin did not proceed to maturity. Because of these degenerative phenomena, the cytoplasm lost its characteristic polychromatic-eosinophilic property to stain faintly basophilic. For other views in regard to the origin of the thrombocytes Mjassojeffoff (14) and Sugiyama (20) should be consulted.

<sup>4</sup> Unpublished data.

## VARIATIONS IN NUMBER OF CELL TYPES IN INDIVIDUAL BLOOD SAMPLES

A review of individual differential counts made from the peripheral blood of embryos from susceptible line 15 showed the blood picture to be fairly constant in all embryos. Only one hemocytoblast was observed; this was found in embryo 50 (line not recorded). One polymorphonuclear heterophilic granulocyte was found in 3 of the 10 differential counts. The maximum number of 5 thrombocytes was found in E894-3 and a minimum of 1 in E894-3 (embryo 2). Thrombocytes were not found in other slides, although 600 or more cells were used for each differential count. Only 2 slides, 50 and E888-2, showed definitive erythroblasts with little hemoglobin; the maximum, 7, was found in 50 and the minimum, 1, in E888-2. A maximum of 23 degenerating cells was counted in E888-1 and a minimum of 3 in E894-1; in 3 of the 9 differential counts no degenerating cells were observed.

## EMBRYOS OF 356-360 HOURS' INCUBATION

## VARIATIONS IN CELL TYPES

A comparison of mean percentages of cell types obtained for embryos of 283-288 hours' incubation with percentages obtained for 356-360 hours' incubation (table 1 and fig. 3) shows that the primitive erythroblasts with much hemoglobin decreased from 6.59 percent in the former to 2.65 percent in the latter, and the definitive erythroblasts with medium hemoglobin decreased from 3.72 percent to 0.91 percent. The definitive erythrocytes increased from 88.29 in embryos of 283-288 hours' incubation to 95.38 in embryos of 356-360 hours' incubation. Variations in size of definitive erythrocytes were also recorded. Immature definitive erythrocytes (fig. 2, *E, se'*) had a mean length of  $10.32\mu$ , a width of  $7.00\mu$ , and an oval nucleus of  $4.27\mu$ ; mature erythrocytes had a length of  $12.54\mu$  and a width of  $6.47\mu$ , with a nucleus  $4.85\mu$  long and  $2.48\mu$  wide. Olson (17), after reviewing the literature, gave the consensus for erythrocyte length as  $12.2\mu$  and width as  $7.3\mu$ .

## VARIATIONS IN NUMBER OF CELL TYPES IN INDIVIDUAL BLOOD SAMPLES

All embryos used for experimentation in the 356-360 hour group were from susceptible line 15. No hemocytoblasts were found although 700-1,200 cells were counted in each of 8 slides. Similarly, other cell types had almost disappeared from the circulation. Only one embryo (E907-2) showed definitive erythroblasts with little hemoglobin (2 of 1,200 cells). In 3 of 8 slides definitive erythroblasts with medium hemoglobin persisted—a maximum of 37 in E893-2 (800 cells counted), 12 in E888-2 (600 cells), and a minimum of 6 in E907-2 (1,200 cells). Polymorphonuclear heterophilic granulocytes decreased. A maximum of 4 granulocytes was found in embryo E907-2 (1,200 cells counted) and a minimum of 2 in 3 of 6 slides, E893-2, E894-6, E907-1 (700 to 900 cells counted). No hemocytoblasts were identified in the peripheral circulation of the 7 embryos. Thrombocytes which showed the characteristic eosinophilic granules were identified in only 1 of the 7 slides.

## EMBRYOS OF 425-431 HOURS' INCUBATION

The mean percentage of definite erythrocytes increased from 95.38 in embryos of 356-360 hours' incubation to 99.16 in embryos of 425-431 hours' incubation (table 1). Primitive erythroblasts with much hemoglobin decreased from a mean percentage of 2.65 to 0.09. No hemocytoblasts or heterophilic granulocytes were found in the peripheral circulation, although a total of 5,600 cells was counted on 5 slides. The mean percentage of thrombocytes increased from 0.21 to 0.68 in embryos of 425-431 hours' incubation. Thrombocytes were found in the peripheral circulation of all embryos. The number was fairly constant. A high count of 10 was recorded for embryo E894-4 and a low of 5 in embryo E1460-1. All embryos used for obtaining the mean percentages of cell types in this age group were from susceptible line 15.

## EMBRYOS OF 475-480 HOURS' INCUBATION

With the exception of thrombocytes, the mean percentages of cell types in embryos of 425-431 hours' incubation and those of 475-480 hours' incubation were essentially the same. Thrombocytes increased from 0.68 in embryos of 425-431 hours' incubation to 1.24 in embryos of 475-478 hours' incubation. Two heterophilic granulocytes were found in E905-1, 3 in E907-2, and none in the remaining 3 embryos. All specimens in this incubation group were obtained from eggs of susceptible line 15.

It is apparent from the data so far presented that the number of blood cells in the peripheral circulation of embryos remains fairly constant from about the seventeenth day until the date of hatching and that the qualitative features of these cells closely approximate those of adult chickens.

## MEAN PERCENTAGES OF CELLS IN THE PERIPHERAL CIRCULATION OF 7 AND 14-DAY INBRED SUSCEPTIBLE AND RESISTANT EMBRYOS

Embryos from inbred susceptible and resistant lines were used exclusively in the following experiments. Thirty-two embryos were obtained from hatching eggs collected from 10 hens from susceptible line 7. Fifteen percent of the breeding stock in this line developed lymphomatosis in 300 days and 23.3 percent in 600 days.

A total of 23 embryos was obtained from hatching eggs produced by 8 hens in resistant line 10. No lymphomatosis, either neutral or visceral, developed in this line in 600 days.

Nineteen embryos were obtained from hatching eggs produced by 7 hens in susceptible line 11. Breeding stock in this line showed 14.4 percent lymphomatosis in 300 days and 45.9 in 600 days.

A total of 44 embryos was obtained from eggs produced by 12 hens in resistant line 13. Breeding stock in this line showed 8.7 percent lymphomatosis in 300 days and 30.4 percent in 600 days.

All blood smears were obtained from the heart of the embryos. The entire liver was taken and prepared for histological study by fixation in Petrunkevitch (18) fixative. Haematoxylin and triosin were used for staining.

In embryos of 161-167 hours' incubation (table 2) the mean percentage of definitive erythrocytes was highest in resistant line 13

TABLE 2.—*Mean percentage of cells of various types in the peripheral blood of resistant and susceptible embryos incubated for 161–167 and 329–339 hours, from various lines of chickens*

Line No.	Em- bryos used	Experi- ment No.	Cells counted	Age of embryos	Primitive erythro- blasts (much hemo- globin)	Prim- itive eryth- rocytes	Little hemo- globin	Definitive ery- throblasts		Heterophilic granulocytes		Mycelioctes		Degen- erating cells	
								Percent		Percent		Percent			
								Defi- nitive ery- throblasts	Medi- um hemo- globin	Hemo- cyto- blasts	Rods	Throm- bocytes	Histo- cytes	Acido- philic	Baso- philic
Line 7 (susceptible)															
Line 10 (resistant)	20	1	8,147	101–107	.97	.97	.97	.44	.44	.12	.01	0	0	0	.01
Line 11 (susceptible)	8	1	4,281	161–167	.75	.75	.75	.37	.37	.02	0	0	0	0	.84
Line 12 (resistant)	2	1	790	164–167	.55	.55	.55	.25	.25	0	0	0	0	0	1.12
Line 13 (resistant)	17	1	12,737	164–167	.34	.34	.34	.30	.30	.07	0	0	0	0	.76
Line 7 (susceptible)	12	2	14,731	329–332	.95	.95	.95	.16	.16	.40	.007	0	0	0	.10
Line 10 (resistant)	15	2	15,313	329–337	.89	.89	.89	.01	.01	.10	0	.006	.96	.007	.007
Line 11 (susceptible)	17	2	19,337	329–338	.84	.84	.84	.03	.03	.03	.01	.006	.86	0	.03
Line 13 (resistant)	27	2	37,723	329–339	.75	.75	.75	.12	.12	.10	.09	.004	.74	0	0

(73.05), next highest in resistant line 10 (65.85), lower in susceptible line 11 (60.37), and lowest in susceptible line 7 (40.46). A further comparison of cell types shows that the mean percentage of definitive erythroblasts with medium hemoglobin was highest (18.31) in the embryos of susceptible line 7 and lowest (1.30) in those of resistant line 10. It was shown earlier in this paper that the cells forming the transitional stages in the development of the erythroblast can be arranged in an orderly sequence with definitive erythroblasts with little hemoglobin at one end and mature definitive erythroblasts at the other. These data suggest that the development of hemopoietic tissue in susceptible line 7 embryos was inhibited to a greater degree than it was in embryos of resistant lines. What was the nature of this inhibition? Obviously, it may be genetic or it may be due to an agent that was transmitted from dam to embryo. Autopsy records show that the incidence of lymphomatosis in line 7 progeny was relatively high (15 percent in 300 days) and took place earlier in life than it did in the progeny from lines 13 and 10 (8.7 and 0, respectively). If this high incidence of lymphomatosis in adult birds was dependent on the introduction of an agent into the egg under natural conditions on or before ovulation, it would follow that further evidence could be obtained either for or against egg transmission by a study of hemopoietic tissues of older embryos from the various lines of chickens.

In embryos of susceptible line 7 incubated for 161–167 hours a total of 10 hemocytoblasts was found in 5 of 20 (8,147 cells counted) blood samples. In line 13 resistant embryos of the same age 52 hemocytoblasts were identified in the peripheral blood of 9 of 17 (12,737 cells counted) embryos. However, in line 10 embryos (161–167 hours' incubation), which were the most resistant of the embryos studied, hemocytoblasts were identified in only 2 of 8 blood slides (4,281 cells). Resistance to pullorum disease in young chicks (19) and to rat sarcoma in chicken embryos (15) has been shown to be rather closely correlated with increase in number of lymphocytes (hemocytoblasts). If the increase in number of hemocytoblasts in the peripheral circulation of embryos of line 13 was a true expression of resistance to lymphomatosis, it would be expected that an increased number of lymphocytes would be maintained in the peripheral circulation of older embryos.

An analysis of the cells in embryos of 329–332 hours' incubation shows that 2 hemocytoblasts were identified in 12 blood samples (14,731 cells counted) from susceptible line 7 embryos, while 6 of 27 smears taken from resistant line 13 embryos contained 37 hemocytoblasts (37,723 cells counted). Embryos from resistant line 10 showed 5 hemocytoblasts in 4 of 15 blood smears (15,313 cells counted), while 1 of the 17 smears made from susceptible line 11 embryos showed a total of 4 hemocytoblasts in 19,337 cells. It is evident from these data and also from data recorded earlier in the paper that the number of hemocytoblasts in some susceptible embryos was about equal to that in some resistant embryos. Obviously, some embryos in susceptible lines are more resistant to lymphomatosis than others or it would be impossible to maintain these lines. In the final analysis, the over-all picture of the differential counts suggests, but does not demonstrate, that resistance to lymphomatosis was expressed in the

embryonal peripheral blood by increased numbers of hemocytoblasts. Hemopoietic variations of this sort differ in many respects from those observed in the peripheral blood of adult chickens infected with naturally occurring lymphomatosis. When the disease developed spontaneously on the laboratory premises, the peripheral blood of some adult chickens showed increased numbers of immature cells of the red cell series while others showed a slight increase in myeloblasts.<sup>5</sup>

MEAN PERCENTAGES OF CELL TYPES IN THE PERIPHERAL CIRCULATION OF EMBRYOS FOLLOWING INJECTION OF THE YOLK SAC WITH TUMOR MINCE AND MINCED NORMAL LIVER

Burmester and Prickett (2) have described a highly malignant strain of tumor developed from naturally occurring avian lymphomatosis; this strain they refer to as RPL-17. In experiment 1 (table 3), 3 gm. of tumorous RPL-17 liver was obtained from donor G1463A under aseptic conditions. The liver was minced, filtered through sterile gauze into a tumor mince bottle containing 9 cc. of NaCl solution (8.5 gm. in 1,000 cc. of distilled water), and the bottle was sealed with a sterile rubber cover. The tumor mince used for inoculation was removed from the bottle with a sterile 24-gage needle and a 2-cc. sterile syringe. All eggs in experiment 1 were incubated for 7 days and then removed from the incubator and washed in dilute heparin solution. Just before inoculation the shell was ground thin at the equator by means of a motor-driven burr, so that the 24-gage needle could be thrust into the yolk sac for inoculating it with tumor mince. Eight eggs were inoculated in this manner with 0.05 cc. of strain 17 tumor mince and 3 with 0.05 cc. of NaCl solution. They were then returned to the incubator along with 15 controls and left for 7 days. On the fourteenth day tumor-mince-injected, NaCl-injected, and control eggs were removed from the incubator and opened. Blood smears and pieces of liver were obtained from individual embryos for further study. All embryos used in the following experiments were from susceptible line 15 with the exception of F1002, F1036, F1040, and F1043, which were from susceptible line 2. Wright and May-Grünwald Giemsa dyes were used for staining blood smears. The livers were fixed in Petrunkevitch (18) fixative and then stained with haematoxylin-triosin.

In experiment 2, the methods used for the injection of tumor (donor G1463A) and NaCl solution were essentially the same as those in experiment 1 except that in experiment 2 injections were made with RPL-17 tumor mince and also with NaCl solution about 24 hours before the eggs were left in the incubator. All eggs were incubated for 14 days before the embryos were sacrificed for the collection of blood smears and liver for histological study. In experiment 2, 11 eggs were injected with 0.05 cc. RPL-17 tumor mince, 11 with normal NaCl solution, and the shells of 3 were punctured but not inoculated. Controls were likewise incubated and studied along with the injected eggs.

In experiment 3 the procedure for inoculation was essentially the same as that already described. Twelve eggs were injected with 0.05 cc. of RPL-17 tumor mince (donor G1269D) and 14 eggs with 0.05 cc. of normal NaCl solution. All inoculations were made about

<sup>5</sup> Personal communication from E. M. Denington.

TABLE 3.—*Mean percentage of cells of various types in the peripheral blood of embryos incubated for 326–338 hours after the yolk sac had been inoculated with RPL-17 tumor mince, grossly normal liver, and normal saline solution*

Embryo No.	Experiment No.	Cells counted	Age of embryo	Primitive erythroblasts (much hemoglobin)		Definitive erythroblasts		Definitive erythrocytes		Heterophilic granulocytes (retro)		Percent 0.57 0
				Hours 320–335	Percent 0.54	Percent 0.15	Percent 2.11	Percent 97.16	Percent 0.03			
F1009, F1017, F1028, F1052, F1056, F1017, F1026, F1043, F1045, F1009, F1017, F1028, F1040, F1029, F1043, F1045, F1028, F1029, F1056.	1	IT <sub>1</sub> C 11,281	Number 6,600 327–331	.57	.06	0	1.13	97.45	0	0	.77	0
F1028, F1040, F1048, F1002, F1009, F1040, F1051, F1028, F1029, F1030, F1040, F1043, F1348.	2	IT <sub>1</sub> S C 8,000	4,900 332–335 332–336 334–337	1.22 .61 .02 .06	.16 .61 .10 0	0 0 0 0	.12 .49 .30 0	97.22 97.70 97.77 0	0 0 0 0	.04 0 .02 0	1.18 1.18 1.13 0	
F1025, F1027, F1033, F1008, F1025, F1028, F1031, F1036.	3	IT <sub>1</sub> S 6,900	5,100 331–334 332–338	.77 .04 .35	0 0 0	0 0 0	.10 .10 .10	98.19 98.73 97.90	0 0 0	.06 .06 .06	.70 .74 .70	
F1004, F1009, F1056-1, F1009, F1028, F1056.	4	IT <sub>1</sub> C 7,000	5,297 327–330 327–331	2.35 .95	1.62 .64 .10	0 0 0	2.07 .31	90.65 97.90	.32 0	.03 0	1.66 .70	.00 .03

<sup>1</sup> IT<sub>1</sub>, 0.05 cc. of RPL-17 tumor mince injected into yolk sac of embryos incubated for 7 days; C, uninjected embryos; IT<sub>1</sub>, 0.05 cc. of RPL-17 tumor mince injected into yolk sac 24 hours before start of incubation; S, 0.05 cc. of saline solution injected 24 hours before start of incubation; IT<sub>1</sub>, 0.05 cc. of grossly normal liver mince injected into yolk sac 24 hours before start of incubation.

24 hours before incubation. Likewise, all eggs were incubated for 14 days before the embryos were used for histological study.

The inoculum employed in experiment 4 was made from the liver of a chicken (donor G1368A) grossly negative for lymphomatosis, from susceptible line 15. Eighteen eggs were inoculated with 0.05 cc. of normal liver—NaCl solution (liver ratio 1:3). Seventeen controls were incubated along with the inoculated eggs for 14 days. The observations made in the four experiments are presented in table 3.

A review of the data given in table 3 shows that the mean percentage differences between the various cell types in RPL-17-injected embryos and their respective controls were so slight that an evaluation of their significance in interpreting the effects of the inoculums on the embryonic hemopoietic system is extremely hazardous.

In experiments 1, 2, and 3, about 45 percent of the injected embryos died within 5 days after inoculation, 2 percent of the eggs were infertile, and 42 percent appeared to be normal in every respect on the fourteenth day of incubation. These experiments showed that normal developmental and physiological processes were maintained in some embryos although known quantities of malignant tumor mince were injected into the yolk sac. Burmester and Prickett (2) maintain that strain 17 tumor mince is similar to the agent of lymphomatosis in several respects. The original inoculum used in these experiments was prepared from lymphomatotic tissues of a chicken that developed lymphomatosis on the laboratory premises. Moreover, the visceral lesions developing after inoculation of strain 17 were grossly and microscopically similar to those found in some cases of naturally occurring visceral lymphomatosis. In view of these facts it seems logical to conclude that under normal conditions the yolk sac may be infected with a tumor-producing agent without any material alteration of the developmental processes of the embryo.

It is evident from experiment 4, table 3, that grossly normal liver inoculum significantly increased the mean percentages of primitive erythroblasts, primitive erythrocytes, hemocytoblasts, thrombocytes, and acidophilic myelocytes and decreased the mean percentage of definitive erythrocytes. Sixty-seven percent of the embryos inoculated with 0.05 cc. of normal liver mince died within 5 days after inoculation. Blount (1) found that pullets after inoculation with liver extract likewise showed significant blood changes (hemocytoblastosis); i. e., hemocytoblasts as well as immature cells of all series were significantly increased. Chilling of young chicks and bacteria also produced analogous hemopoietic variations. Jungherr (13), after summarizing the literature on blood changes in the avian leukosis complex, concluded that in some forms of lymphomatosis (ocular and neural) no significant alterations take place in the blood picture, while in other forms of lymphomatosis (visceral) specific blood changes, either qualitative or quantitative in nature, are of frequent occurrence. Qualitative variations are either temporary or terminal, and less constant than those in erythroblastosis or granuloblastosis. On the basis of the observations given in the foregoing review, it seems fairly safe to conclude that hemocytoblastosis as the only criterion for diagnosis of lymphomatosis in adult chickens should be used with considerable reserve. Likewise, the use of hemocytoblastosis as a diagnostic criterion for ascertaining whether or not lymphomatosis is

egg-borne seems to be of limited value, since RPL-17 tumor mince produced no detectable blood changes in the peripheral circulation of surviving embryos. Nevertheless, liver mince prepared from grossly negative tissues did produce blood changes in the embryo which in a descriptive sense met the requirements for hemocytoblastosis.

#### DISCUSSION

In the preceding pages it has been shown that variations in number of hemocytoblasts and other cell types in the peripheral circulation of inbred resistant and susceptible embryos are fairly common. Obviously, there may be several interpretations of such expressions of differences in the physiological condition of the hemopoietic foci of the embryo; for example, (1) the increased numbers of hemocytoblasts in the peripheral circulation of embryos may indicate the reactivity of the hemopoietic tissues to the agent of lymphomatosis; (2) resistance to lymphomatosis in inbred chickens may be expressed in the embryo by increased numbers of hemocytoblasts in the embryonal peripheral circulation; and (3) a close correlation may exist between retardation in embryonic development and increased numbers of hemocytoblasts in the embryonic peripheral circulation.

If lymphomatosis is an egg-borne disease, transmitted from dam to offspring by means of a filterable agent or tumor cell, embryonic tissues other than peripheral blood would be expected to show manifestations of the disease. Embryos exhibiting increased numbers of hemocytoblasts were of normal size and there were no gross or microscopic evidences of infiltration or proliferation of lymphocytes in the liver or of abnormal eye conditions comparable to those found in infected adult birds. Furthermore, there was no evidence of embryonic fowl paralysis, since movement of appendages and other body parts of embryos seemed to be normal in every respect. These observations suggest that if lymphomatosis is egg-borne, there are no gross pathological manifestations of the disease in the embryo.

Assuming that genetic resistance to lymphomatosis is expressed in the embryo by increased numbers of hemocytoblasts in the embryonal peripheral circulation, it is to be expected that some embryos in the susceptible lines will show increased numbers of hemocytoblasts, since these lines are resistant enough to supply breeding stock and sufficient numbers of chickens for experimentation. Evidence for such a differential resistance to lymphomatosis was presented in the discussion of variations in differential counts of the peripheral blood of embryos incubated for 7 and 14 days, from lines 7, 10, 11, and 13. These differences may be due to genetic heterozygosity of resistant and susceptible lines of chickens. At the present time, the various lines of chickens used for experimentation at this laboratory are not genetically stabilized either for resistance or susceptibility. Resistance to lymphomatosis in resistant lines 10 and 13 and susceptible lines 7 and 11 is expressed statistically. If and when resistance to lymphomatosis becomes more firmly established on a genetic basis, it may be possible to correlate more completely increased numbers of hemocytoblasts in the peripheral circulation with resistance to lymphomatosis.

The third explanation suggested above for increased numbers of hemocytoblasts in the peripheral circulation of embryos takes into

consideration the possible effects of retardation in embryonal development. It has been rather well established in this laboratory that embryos from certain lines of chickens show a significant developmental lag, i. e., the actual age of embryos when estimated by somite count is frequently found to be from 3 to 20 hours less than the chronological age. This discrepancy between somite age and chronological age is well illustrated by a comparison of line 7 susceptible embryos with line 10 resistant embryos incubated for 161-167 hours (table 2). The mean percentage of definitive erythrocytes in the susceptible embryos was .40.46, while the mean percentage in the resistant embryos was 65.85. The fact that the mean percentage of definitive erythroblasts with medium hemoglobin was significantly higher in susceptible embryos (18.31) than in resistant embryos (1.30) definitely establishes this developmental lag, since an analysis of the data in table 1 shows that an increase in number of definitive erythrocytes is followed by a corresponding decrease in definitive erythroblasts. The data given in the preceding pages show also that 5 of 20 embryos in line 7 (161-167 hours) had only 10 hemocytoblasts. Thus, a correlation of embryonal developmental lag with number of hemocytoblasts is untenable.

#### SUMMARY

Two generations of blood cells, the primitive and definitive, were found in the peripheral circulation of chicken embryos.

Primitive erythroblasts and hemocytoblasts were predominant cell types of the peripheral circulation until about the seventh day of incubation. Other cells were occasionally found, e. g., histiocytes, basophilic and acidophilic myelocytes, giant cells, thrombocytes, polymorphonuclear neutrophilic leucocytes, and degenerating cells.

On about the seventh day of incubation, significant numbers of cells of the definitive generation appeared in the peripheral circulation. The most immature erythroblast of the definitive series contained less hemoglobin and was smaller than the most immature erythroblast of the primitive series.

Giant cells were occasionally found in supravital preparations of the peripheral blood of embryos of various ages, and these cells frequently gave rise to one or more granulocytes by the production of cellular blebs. Such blebs ultimately pulled away from the surface and became free. Giant cells under other conditions fragmented to form thrombocytelike cells.

Hemopoietic tissue in the peripheral blood of embryos approached stability on about the ninth day of incubation. The mean percentage of primitive erythroblasts at about this time decreased to 5.14 while the mean percentage of definitive erythrocytes showed a corresponding increase to 90.22.

The frequency of occurrence and also the total number of hemocytoblasts in the peripheral circulation of embryos from resistant lines 13 and 10 were higher than in that of embryos from susceptible lines 7 and 11. The evidence suggests, but does not demonstrate, that increased numbers of hemocytoblasts in the peripheral circulation may be an important factor in resistance to lymphomatosis. Other resistant factors were probably operative, since embryos receiving RPL-

17 tumor mince by yolk-sac injection showed a peripheral blood picture which was essentially the same as that of uninjected control embryos.

The peripheral blood of resistant embryos with higher than normal numbers of hemocytoblasts differed markedly from that of adult chickens with lymphomatosis. In the former, all blood cells other than hemocytoblasts were essentially normal morphologically as well as numerically; in the latter, the peripheral blood of some infected adult birds frequently showed increased numbers of immature cells of the red cell series while that of others showed increased numbers of myeloblasts.

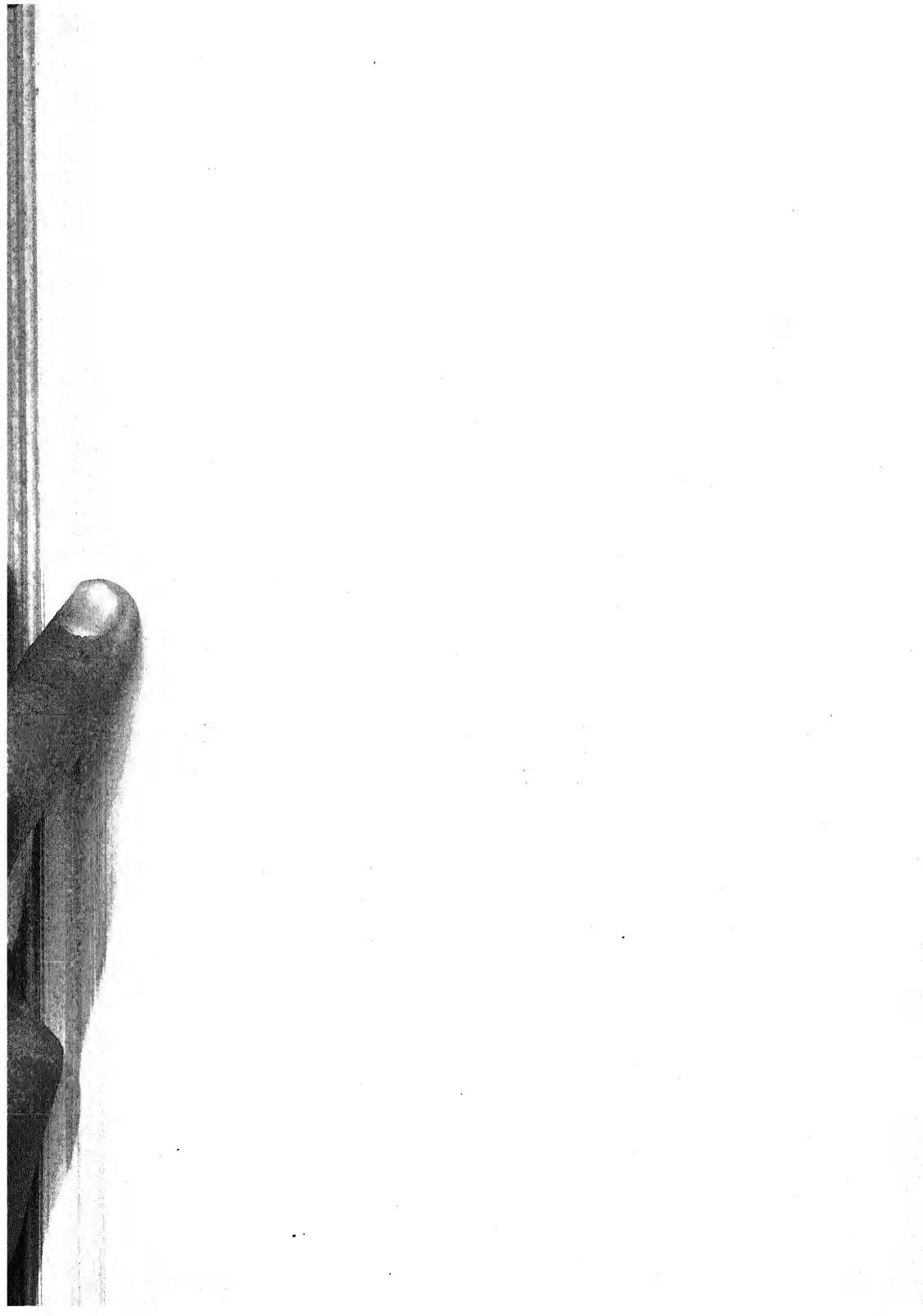
The mean percentage of definitive erythrocytes in embryos of 161-167 hours' incubation was highest in resistant line 13 (73.05), next highest in resistant line 10 (65.85), lower in susceptible line 11 (60.37), and lowest in susceptible line 7 (40.46).

Normal liver injected into the yolk sac prior to incubation significantly increased the mean percentages of primitive erythroblasts, primitive erythrocytes, hemocytoblasts, thrombocytes, and acidophilic myelocytes and decreased the mean percentage of definitive erythrocytes. Sixty-seven percent of the embryos receiving grossly normal liver mince died within 5 days after inoculation.

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## INHERITANCE OF REACTION TO RUST IN FLAX<sup>1</sup>

By H. H. FLOR

*Pathologist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture*

### INTRODUCTION

All varieties of cultivated flax tested have been found to be susceptible to one or more of the races of flax rust (*Melampsora lini* (Pers.) Lév.) occurring naturally in North America or South America (3, 10).<sup>2</sup> Although no variety that is resistant to all races is known, it may be possible to develop such a variety by combining through hybridization various resistant or immune factors possessed by certain varieties that serve to differentiate the physiologic races. Races capable of attacking all varieties are not known in any one flax-producing region; therefore it is not yet necessary to incorporate resistance to all known races into a variety developed for a particular region. Nevertheless, a program to develop rust-resistant varieties of flax would be facilitated by knowledge of (1) the pathogenic capacities of the physiologic races of *M. lini* occurring or likely to occur in the region with which the investigator is concerned and (2) the interaction between the factors conditioning resistance to or immunity from rust. The present paper is concerned primarily with the latter phase of such a program.

### REVIEW OF LITERATURE

Henry (7) found that immunity from a North American rust collection was dominant and conditioned by single pairs of factors in Ottawa 770B and Bombay and by 2 pairs in an Argentine selection. In a study on the inheritance of reaction to North American races of rust in crosses involving 17 varieties of flax, Myers (8) accounted for his results by assuming factors in 2 allelic series. Immunity was dominant and conditioned by the duplicate factors *L* and *M*. The less resistant infection types were hypostatic to the more resistant ones, the factors *l<sup>n</sup>* and *m<sup>n</sup>* conditioning near immunity and *l<sup>r</sup>* and *m<sup>r</sup>* resistance. Susceptibility was conditioned by the recessive factors *ll* and *mm*. In a study on the inheritance of rust reaction in a cross between Buda and J. W. S., Flor (4) found that each of these varieties possessed a pair of factors which conditioned immunity from certain

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<sup>2</sup> Italic numbers in parentheses refer to Literature Cited, p. 262.

races of rust and were allelomorphic to the factors for immunity in Ottawa 770B. He also found that a single pair of factors conditioned resistance to one race, semiresistance to a second, moderate susceptibility to a third, and high susceptibility to a fourth.

In studies on the inheritance of factors conditioning resistance to disease in plants, it usually has been assumed that the two parent varieties have identical factors for resistance when their hybrid progeny yield no segregates susceptible to a race of the pathogen to which both parents are resistant (8, 9). However, this may not necessarily be true if the factors are allelomorphic. Myers (8) considered the immune factors possessed by Ottawa 770B and C. I.<sup>3</sup> 438 (selection Minn. 25-107 from Minn. 281) to be identical. Flor (3) subsequently found that C. I. 438 was highly susceptible to races 19 and 20 of *Melampsora lini*, whereas Ottawa 770B was immune from these races. Ottawa 770B and C. I. 438 apparently possess duplicate allelomorphic factors for immunity from certain North American races.

#### MATERIALS AND METHODS

It was originally planned to study the inheritance of rust reaction in flax (*Linum usitatissimum L.*) by crossing in all possible combinations the 11 varieties used to differentiate physiologic races of rust (3). During the later phases of the study hybrid rust cultures (6) became available; by use of these, 6 varieties from the group that had been classed as immune from or resistant to North American races and susceptible to South American races (3) were found to possess rust-conditioning factors distinct from each other and from those of the 11 rust differentials. The varieties Newland, Bolley Golden, Italia Roma, Leona, and Tammes Pale Blue had sharp differences between resistant or immune and susceptible reactions and have been included in this study. The reaction of the sixth variety, Light Mauve (C. I. 379-1), although distinct from each of the others, was one of degree and subject to considerable variation by changes in environment. Because of this variability, a genetic study of the factors for rust reaction possessed by this variety was not undertaken. In addition, limited studies were made on the inheritance of rust-conditioning factors in C. I. 416-3 (Pale Verbena) and C. I. 438, varieties studied by Myers (8), in Rio, a variety developed at the North Dakota Agricultural Experiment Station that has been immune from North American races of flax rust, and in Billings.

Except for the selection of Tammes Pale Blue, which had an occasional offtype plant, the differential varieties were pure for rust reaction when tested with the 24 races that had been identified from North American and South American collections of the pathogen. However, tests with  $F_2$  cultures of hybrids of North American race 24 with South American race 22 showed that Argentine selection (C. I. 462) was homozygous for 1 pair of factors conditioning immunity from the known North American races and susceptibility to South American races 19, 20, and 22 but probably heterozygous for 2 other pairs of factors. Extensive tests with the  $F_2$  hybrid rust cultures indicated that Burnham's selection of Morye (Argentine, C. I. 112) (1) was

<sup>3</sup>C. I. refers to accession number of the Division of Cereal Crops and Diseases.

homozygous for these 3 pairs of rust-conditioning factors. Consequently, Morye has been substituted for C. I. 462 as a rust differential.

Data presented in this paper are the result of tests of the  $F_2$  populations. Flax is adapted for such a procedure; under favorable conditions each plant produces several hundred seeds so that statistically significant results were usually obtained from the progeny of a single  $F_1$  plant. Most of the rust-differentiating varieties of flax have sharp differences in reaction to the various physiologic races of the pathogen, being highly susceptible, highly resistant, or immune. This reduced the errors in reading the reaction of individual  $F_2$  plants attributable to the effects that changes in environment exert on the expression of the intermediate infection types. Finally, the reaction of most of the differential varieties of flax to rust is inherited in a relatively simple manner, being conditioned by one, two, or three pairs of major factors.

These tests were conducted in the pathology greenhouse at the North Dakota Agricultural Experiment Station, Fargo, N. Dak., during the late fall, winter, and early spring. The usual test of 192  $F_2$  seeds gave sufficient plants for a statistical analysis of the results in crosses involving as many as 3 pairs of rust-conditioning factors. Eight seeds were sown in each 4½-inch pot in Fargo clay topsoil that had been steamed for 2 hours at 15 pounds' pressure. Because flax grows by elongation of the terminal bud it was possible to test each plant with as many as 5 physiologic races. This was done by inoculating the unfolding leaves of the terminal bud with 1 race when the seedlings were 2 to 3 inches tall and repeating the inoculation with additional races at 7- or 8-day intervals. As soon as the rust had developed sufficiently to differentiate the type of pustule, usually 9 to 10 days after inoculation, the type was recorded and the plant tagged with colored yarn representing a definite reaction to a specific race. The infected leaves were then picked off, leaving the terminal bud and apical leaves bearing the subsequent inoculation. When races having the required pathogenic capacities were available, the usual procedure was to inoculate the  $F_2$  plants first with a race to which both parents were resistant, then with a race to which one parent was resistant and the other susceptible, and finally with a race to which the parents had the reverse reactions.

Plants were classified according to rust reaction on the basis of pustule type as previously described (2, 4).

Immunity has invariably been dominant to resistance, and high resistance has usually been dominant to low. In some varieties the dominance of resistance is incomplete, plants heterozygous for a pair of rust-conditioning factors being less resistant to certain races than homozygous plants (4).

In the earlier studies the only races of rust used had been isolated from field collections. Consequently, in a number of crosses races capable of attacking one differential parent of the hybrid but unable to attack the other were not available. As a result of studies on the inheritance of pathogenicity in crosses between races 6 and 22 and races 22 and 24 (6), races of hybrid origin having almost all possible combinations of virulent and avirulent factors were obtained and used in the later studies.

TABLE I.—*Inheritance of factors for rust reaction in flax varieties as determined by the reaction of the  $F_2$  progeny to races of *Melampsora lini*, to which one parent was resistant or immune and the other susceptible*

C. I. No.	Differential parent and reaction to indicated race			Susceptible parent			$F_2$ plants having indicated reaction <sup>1</sup>			Number of pairs of factors	Theoretical ratio	$\chi^2$ value	P between <sup>2</sup>	
	Parent	Reaction <sup>1</sup>	Race				I	R	S					
				Number	Number	Number	I	R	S					
355	Ottawa 70B	I	Bison	16	95	0	33	1	3:1	0.035	0.80 and 0.90.			
188	Bombyx	I	Whiona	21	137	0	46	1	3:1	.019	.100.			
42	Williston Brown	I	Bison	1	151	0	38	1	3:1	.205	0.10 and 0.20.			
803-1	Akmolinsk	R	do	52	0	143	45	1	3:1	.114	0.70 and 0.80.			
515-1	J. W. S.	R	do	1	0	144	48	1	3:1	.000	( <sup>3</sup> ).			
708-1	Pale Blue Crimped	R	do	1	113	0	43	1	3:1	.547	0.30 and 0.50.			
647	Kenya	R-	do	24	0	3 100	26	1	3:1	.280	0.20 and 0.30.			
709-1	Abyssinian	R-	do	32	0	0	3 136	56	1	3:1	1.777	0.10 and 0.20.		
701	Leona	R	do	1	149	0	43	1	3:1	.605	0.30 and 0.50.			
836	Newland	R	do	836	0	148	43	1	3:1	.635	.700.			
338-1	Tamms Pale Blue	R	do	9	J. W. S.	131	0	33	1	3:1	.081	0.10 and 0.20.		
184	Billinggs.	I	Bison	24	do	139	0	41	1	3:1	.473	0.36 and 0.50.		
270-1	Bella	I	do	7	do	234	57	19	2	12:3:1	.010	0.90 and 0.95.		
28-1	Williston Golden	R	do	7	do	146	10	2	15:1	.083	0.80 and 0.99.			
644	Bolley Golden	I	do	24	do	125	3 41	5	5:11	0.05 and 0.10.				
1005-2	Italia Roma	I	do	24	do	140	3 38	14	2	12:3:1	.555	0.30 and 0.50.		
438	Minn. 25-107	R	do	24	do	0	3 297	23	4:2	.480	Do.			
416-3	Pale Verbena	I	do	4	do	5 178	0	7	4:2	.151	1.920	0.10 and 0.20.		
112	Morye	I	do	24	do	375	0	8	3	63:1	.668	0.30 and 0.50.		
280	Rio	I	do	21	do	300	0	6	3	63:1	.360	0.50 and 0.70.		
280	do	I	do	21	J. W. S.	266	0	3	3	63:1	.022	0.80 and 0.90.		

<sup>1</sup> I, immune; R, resistant; R-, moderately resistant; S, susceptible.

<sup>2</sup>  $I_2$ .

<sup>3</sup> Reaction of plants placed in this category somewhat variable, possibly because of the sensitivity of the intermediate infection types to differences in environment and to host vigor.

<sup>4</sup> These strains were not pure; some plants had only 1 hair of rust-conditioning factors.

<sup>5</sup> Some resistant plants included.

## EXPERIMENTAL RESULTS

## CROSSES BETWEEN DIFFERENTIAL RESISTANT AND CERTAIN SUSCEPTIBLE VARIETIES

Results obtained from tests to determine the number of factors for resistance to or immunity from rust possessed by each of the flax varieties studied are given in table 1. In these tests each  $F_2$  progeny was inoculated with one or more races to which the differential parent was resistant and the other parent susceptible. These data show that resistance to or immunity from rust is dominant and conditioned by single pairs of factors in 12 of the varieties studied, by 2 factors in 6, and by 3 factors in 2.

It is possible that some of the differential flax varieties possess additional factors for rust resistance that were not effective against the races used in these studies. Consequently, the number of rust-conditioning factors possessed by each variety, as given in table 1, should be regarded as a minimum figure. Waterhouse and Watson (12), who found Bison to be immune from the races of flax rust occurring in their tests in Australia, used Bombay, a variety immune from South American and most North American races, as their susceptible host. Obviously, Bison would not have served as a susceptible parent if those particular Australian races had been used in the present studies.

Tests of  $F_2$  of Bison  $\times$  Buda and Bison  $\times$  Williston Golden with various races (table 2) illustrate the need for determining the pathogenic

TABLE 2.—*Parental reaction to various races of Melampsora lini and segregation for reaction in  $F_2$  plants of crosses of Bison  $\times$  Buda and Bison  $\times$  Williston Golden*

Cross and race tested	Reaction <sup>1</sup> to indicated race of—						$\chi^2$ value	P between—	
	Parent listed first	Parent listed second	$F_2$ plants						
<b>Bison <math>\times</math> Buda:</b>									
7.....	S	I	I	I	R	S	.....	.....	
5.....	S	R	R	R	R-	S	.....	.....	
3.....	S	SR	SR	S- to S	SR	S	.....	.....	
20.....	S	I	I	I	S	S	.....	.....	
Plants observed.....			188	46	57	19	3.622	0.30 and 0.50.	
Plants expected (9:3:3:1).....			175	58	58	19			
<b>Bison <math>\times</math> Williston Golden:</b>									
7.....	S	R	R	R	R	S	.....	.....	
52.....	S	R	R	R	S	S	.....	.....	
16.....	S	R	R	S	R	S	.....	.....	
Plants observed.....			92	26	28	10	.625	0.80 and 0.90.	
Plants expected (9:3:3:1).....			88	29	29	10			

<sup>1</sup> I, immune; R, resistant; R-, moderately resistant; SR, semiresistant; S-, moderately susceptible; S, susceptible.

capacities of the races used as well as for reserving judgment as to the finality of the results. The segregation of  $F_2$  plants of Bison  $\times$  Buda for reaction to the races tested can be explained by assuming that immunity from or resistance to rust was dominant and that Bison carried no rust-conditioning factors and Buda two pairs. Both pairs were effective in conditioning resistance to race 5; one was almost inoperative against race 3, but conditioned immunity from races 7 and

20; the second pair was inoperative against race 20, but conditioned semi-resistance to race 3 and resistance to race 7.

Segregation for rust reaction in the  $F_2$  population of Bison  $\times$  Williston Golden to races 7, 52, and 16 of *Melampsora lini* illustrates the specific relation that exists between the different races of the pathogen and the individual factors conditioning rust resistance in the host. The parent varieties reacted the same to each of these 3 races, Bison being susceptible and Williston Golden resistant. The segregation of  $F_2$  plants for reaction to race 7 of 146 resistant to 10 susceptible approximated a 15:1 ratio, suggesting that resistance to race 7 in Williston Golden was conditioned by 2 pairs of duplicate factors. The 10  $F_2$  plants susceptible to race 7 were susceptible also to races 52 and 16. Of the 146  $F_2$  plants resistant to race 7, 92 were also resistant to races 16 and 52, 28 were resistant to race 16 and susceptible to race 52, and 26 were resistant to race 52 and susceptible to race 16. This ratio of segregates for reaction to each race approximates that expected if rust resistance is a dominant character and Williston Golden possesses 2 pairs of rust-conditioning factors, of which one is operative against race 52 and the other against race 16 and both condition resistance to race 7.

#### CROSSES BETWEEN DIFFERENTIAL VARIETIES

As the study progressed, it became apparent that most of the factors for rust reaction lay in the three allelomorphic series or linkage groups represented by the factors occurring in Ottawa 770B, Newland, and Bombay. Rust reaction in each of these varieties apparently is conditioned by a single pair of independently inherited factors. Ottawa 770B, Newland, and Bombay have been either immune from or highly susceptible to every physiologic race with which they have been tested, suggesting that secondary rust-conditioning factors are absent. In later phases of these investigations inheritance of rust reaction in the  $F_2$  population of hybrids of the variety being studied with each of these three varieties was first determined. The lack of segregates susceptible to races to which both parents were immune or resistant was interpreted as indicating the presence of a rust-conditioning factor in the variety being studied which was allelomorphic to or linked with the factor for immunity in Ottawa 770B, Newland, or Bombay as the case might be. The occurrence of appropriate numbers of susceptible segregates indicated lack of allelomorphism or linkage.

#### CROSSES INVOLVING OTTAWA 770B

Of the 11 differential varieties in which rust reaction is conditioned by a single pair of factors, 4 have factors that lie in the Ottawa 770B allelomorphic series (table 3). These varieties, in addition to Ottawa 770B, are J. W. S., Pale Blue Crimped, and Kenya. One of the 2 pairs of rust-conditioning factors in Buda is allelomorphic to the Ottawa 770B factor, as is 1 of the 2 in Williston Golden. Both pairs of rust-conditioning factors possessed by Bolley Golden and Italia Roma are inherited independently of the pair in Ottawa 770B.

None of the 754  $F_2$  plants of the cross Morye  $\times$  Ottawa 770B was susceptible to race 24, which attacked neither parent; this indicates an allelic relation between 1 of the 3 rust-conditioning factors in Morye and that in Ottawa 770B. Since some plants were susceptible to both

TABLE 3.—*Parental reaction to various races of Melampsora lini and segregation of F<sub>2</sub> plants of crosses involving Ottawa 770B*

Cross and race tested	Reaction <sup>1</sup> to indicated race of—					$\chi^2$ value	$t$ <sup>2</sup> be- tween—
	Parent listed first	Parent listed second	F <sub>2</sub> plants				
Ottawa 770B × New- land:			I	I	S	S	
7----- 20-----	I I	I S	I I	I S	11 12		
Plants observed.			147	34			
Plants expected (12:3:1).			144	36			0.257
Ottawa 770B × Born- bay:			I	I	I	S	
3----- 22----- 24-----	I S I	I I S	I I I	I S I	S S S		
Plants observed.			110	43	32	9	
Plants expected (9:3:3:1).			109	36	36	12	2.563
Williston Brown × Ott- awa 770B:			R	I	I	S	
52----- 7----- 47----- 16-----	R R R S	I I R I	I I I I	R R R S	S S S S		
Plants observed.			103	35	39	15	
Plants expected (9:3:3:1).			108	36	36	12	1.259
Ottawa 770B × Akmo- llink:			I	R	S		
2----- 7-----	I I	R S	I I	R S	S		
Plants observed.			101	22	5		
Plants expected (12:3:1).			96	24	8		1.552
Ottawa 770B × J. W. S.:			I	I	I	S	
19----- 7----- 22-----	I S I	I S I	I I I	I S I	S S S		
Plants observed.			95	45	48	0	
Plants expected (2:1:1:0).			94	47	47	0	.117
Ottawa 770B × Pale Blue Crimped:			I	R	R and I	S	
7-----	I	R	124	0			
Plants observed.			124	0			(?)
Plants expected (1:0).							(?).
Kenya × Ottawa 770B:			R	I	I	S	
7----- 68----- 79-----	R R S	I S I	I R I	I S I	S S S		
Plants observed.			35	13	16	0	
Plants expected (2:1:1:0).			32	16	16	0	.844
Ottawa 770B × Abys- sinian:			I	I	I	S	
1----- 3----- 8-----	I I I	R S I	I I I	R S S	S S S		
Plants observed.			137	45	18		
Plants expected (12:3:1).			150	38	12		5.047
Leona × Ottawa 770B:			R	I	R	S	
24----- 68----- 52-----	R R S	I S I	R R I	R S I	S S S		
Plants observed.			113	38	27	6	
Plants expected (9:3:3:1).			104	34	34	12	5.487
Ottawa 770B × Tammes Pale Blue:			I	I	I	S	
16----- 20-----	I I	S S	I I	I S	S S		

See footnotes at end of table.

TABLE 3.—*Parental reaction to various races of *Melampsora lini* and segregation of F<sub>2</sub> plants of crosses involving Ottawa 770B—Continued*

Cross and race tested	Reaction <sup>1</sup> to indicated race of—						χ <sup>2</sup> value	P between—		
	Parent listed first	Parent listed second	F <sub>2</sub> plants							
			I	I	R to SR	S				
Plants observed			146	30	14					
Plants expected (12:3:1).			142	36	12		1.356	0.50 and 0.70.		
Ottawa 770B × Buda:										
20	I	I								
3	I	SR	I							
21	I	S	I	S	S	S				
Plants observed			148	35	14	0				
Plants expected (12:3:1:0).			148	37	12	0	.333	0.80 and 0.90.		
Williston Golden × Ott- awa 770B:										
52	R	I	I	I	R	S				
47	R	S	R	S	R	S				
16	R	I	I	I	R	S				
24	S	I	I	I	S	S				
Plants observed			112	37	31	12 0				
Plants expected (9:3:3:1:0).			108	36	36	12 0	.870	0.80 and 0.90.		
Ottawa 770B × Bolley Golden:										
16	I	I	I	R						
19	I	S	I	S		S				
Plants observed			237	49	19	4				
Plants expected (48:12:9:3:3:1).			232	58	14	5	3.047	0.30 and 0.50.		
Italia Roma × Ottawa 770B:										
24	I	I	I	I	I	R				
68	I	S	I	I	R—	S—				
105	S	I	I	S	I	I				
Plants observed			98	31	24	12 10 3				
Plants expected (36:12:9:3:3:1).			100	33	25	8 8 3	2.210	0.80 and 0.90.		
Morge × Ottawa 770B:										
24	I	I	I	I	I	I				
68	I	S	I	I	S	S				
75	S	I	I	S	I	S				
Plants observed			485	183	70	16 0				
Plants expected (45:15:3:1:0).			530	177	35	12 0	39.375	(%).		
Billings × Ottawa 770B:										
24	I	I	I	I	I	S				
68	I	S	I	I	S	S				
85	S	I	I	S	I	S				
Plants observed			114	33	28	7				
Plants expected (9:3:3:1).			103	34	34	11	4.138	0.20 and 0.30.		
C. I. 438 × Ottawa 770B:										
7	R	I	I	I	S					
1	R—	I	I	R—	S					
20	S	I	I	S	S					
Plants observed			137	45	0					
Plants expected (3:1:0).			136	46	0		.007	0.90 and 0.95.		
C. I. 416-3 × Ottawa 770B:										
41	I	I	I	I	S					
20	S	I	I	S	S					
Plants observed			263	76	25					
Plants expected (12:3:1).			277	69	23		1.176	0.50 and 0.70.		
Ottawa 770B × Rio:										
16	I	I	I	I	R	S				
20	I	S	I	S	S	S				
Plants observed			223	64	5	0				
Plants expected (48:15:1:0).			219	68	5	0	.403	0.80 and 0.90.		

<sup>1</sup> I, immune; R, resistant; R—, moderately resistant; SR, semiresistant; S—, moderately susceptible; S, susceptible.<sup>2</sup> No segregation to races tested.<sup>3</sup> P, <0.01.

race 68 and race 75 it must be assumed that the allelomorphic factors are inoperative against race 68. However, the occurrence of 70 segregates immune from races 24 and 75 and susceptible to race 68 cannot be satisfactorily explained by assuming that immunity from the last-named race is conditioned by either or both of the nonallelomorphic rust-conditioning factors in Morye. This number of segregates approximates that expected if race 68 were a mixture of 2 races, one of which was virulent on Ottawa 770B and on plants carrying the rust-conditioning factor in Morye allelic to that in Ottawa 770B and the other virulent on Ottawa 770B and on plants carrying one of the nonallelic rust-conditioning factors possessed by the Morye parent. However, methods usually employed successfully in isolating the components of race mixtures have failed to indicate that race 68 is not pure.

The C. I. 438 parent was heterozygous; in some crosses it contributed two rust-conditioning factors and in others one. In the cross C. I. 438 × Ottawa 770B the single factors in each variety were allelic. The C. I. 438 factor is probably distinct from those possessed by any of the differential varieties.

One of the three factors in Rio also lies in the Ottawa 770B allelomorphic series. Although this factor in Rio may be distinct, at the present time there is no experimental basis for separating it from the Morye factor.

#### CROSSES INVOLVING NEWLAND

In every cross involving Newland (table 4) the  $\chi^2$  value for fit of the observed to the expected ratio of segregates gave a  $P$  of more than 0.10, indicating agreement of the data with the hypotheses and regularity in segregation of the Newland factor. The observed ratios may be explained by assuming that the single pair of rust-conditioning factors in Williston Brown and one of the two in Buda, Williston Golden, Bolley Golden, and Italia Roma are allelic to the pair in Newland. The rust-conditioning factors in the other differential varieties, including all three pairs in Morye, are inherited independently of the Newland factor. The single pair in Billings is allelic to that in Newland and one of the three in Rio may be, but the number of plants studied was not sufficient to give statistically significant results in a hybrid involving four factors.

Myers (8) concluded that a near-immune factor allelic to that in Newland conditioned reaction in C. I. 416-3 to the races he used. The parent material used in the present study apparently was heterozygous, since the ratios of  $F_2$  segregates in C. I. 416-3 × Bison (table 1) and C. I. 416-3 × Bombay (see table 5) may be explained by assuming that C. I. 416-3 carried two rust-conditioning factors, whereas the segregation ratios of the  $F_2$  hybrids of C. I. 416-3 with Ottawa 770B (table 3) and Newland (table 4) indicate that each parent contributed but a single rust-conditioning factor. In the cross C. I. 416-3 × Newland, no segregates susceptible to race 16, from which both parents are immune, were obtained; this substantiated Myers' conclusions that in these varieties rust-conditioning factors are allelic. The  $F_2$  plants segregated into a ratio approximating 3 immune to 1 not immune, the latter ranging in reaction from necrosis of the inoculated tissue to moderate susceptibility. Since the Newland factor has invariably conditioned immunity from race 16, it is probable that modifying factors

TABLE 4.—*Parental reaction to various races of Melampsora lini and segregation of F<sub>2</sub> plants of crosses involving Newland*

Cross and race tested	Reaction <sup>1</sup> to indicated race of—						χ <sup>2</sup> value	P between—		
	Parent listed first	Parent listed second	F <sub>2</sub> plants							
			(2)	(2)	(2)	(2)				
Ottawa 770B × Newland	(2)	(2)	I	I	I	S				
Bombay × Newland:	I	I	I	I	S	S				
1.....	I	S	I	I	S	S				
19.....	I	I	I	I	S	S				
24.....	I	I	I	S	I	S				
Plants observed.....			104	36	40	12				
Plants expected (9:3:3:1).....			108	36	36	12				
Williston Brown × Newland:	R	I	I	I	R	S				
7.....	R	S	I	I	R	S				
47.....	R	I	I	I	R	S				
16.....	R	S	I	S	R	S				
Plants observed.....			83	38	38	0				
Plants expected (2:1:1:0).....			79	40	40	0				
J. W. S. × Newland:	I	I	I	I	I	S				
1.....	I	S	I	I	S	S				
9.....	I	S	I	S	I	S				
19.....	I	S	I	S	I	S				
Plants observed.....			104	35	43	10				
Plants expected (9:3:3:1).....			108	36	36	12				
Kenya × Newland:	R	I	I	I	R	S— to S				
24.....	R	I	I	S—	R— to S	S— to S				
81.....	R	S	R	S—	R—	S— to S				
73.....	R	S	R	S—	R—	S— to S				
Plants observed.....			113	32	32	7				
Plants expected (9:3:3:1).....			104	34	34	12				
Newland × Leon:	I	R	I	I	R	S				
24.....	I	S	I	I	S	S				
52.....	I	R	I	S	R	S				
85.....	S	R	I	S	R	S				
Plants observed.....			114	26	35	17				
Plants expected (9:3:3:1).....			108	36	36	12				
Newland × Tamms Pale Blue:	I	I	I	S						
1.....	I	I	I	S						
Plants observed.....			175	7						
Plants expected (15:1).....			171	11						
Buda × Newland:	I	I	I	I to R	I	S				
7.....	SR	I	I	R to SR	I	S				
3.....	S	I	I	S	I	S				
4.....	R	S	R	R	S	S				
111.....	R	S	R	R	S	S				
Plants observed.....			158	51	13	0				
Plants expected (11:4:1:0).....			153	55	14	0				
Williston Golden × Newland:	R	I	I	R	I	R				
7.....	R	I	I	R	I	R				
16.....	R	I	I	R	I	R				
52.....	R	S	R—	R	S	R				
47.....	R	S	I	S	I	S				
68.....	R	S	I	S	I	S				

See footnotes at end of table.

TABLE 4.—*Parental reaction to various races of Melampsora lini and segregation of F<sub>2</sub> plants of crosses involving Newland—Continued*

Cross and race tested	Reaction <sup>1</sup> to indicated race of—						$\chi^2$ value	P between		
	Parent listed first	Parent listed second	F <sub>2</sub> plants							
			I	S	I	S				
Plants observed. Plants expected (11:3:1:1:0).			95	28	14	13	0	4.325	0.29 and 0.30.	
103	28	9	9	0						
Newland × Bolley Golden:										
16	I	I								
41	I	I	I	S						
Plants observed. Plants expected (1:0).			192	0				{ (?)	{ (?)	
192	0									
Italia Roma × Newland:										
24	I	I	I	I	I	I	S			
60	I	S	I	I	S	S	S			
52A	R-	I	I	I	R- to S	I	I			
Plants observed. Plants expected (8:4:3:1:0).			101	48	32	10	0	1.156	0.70 and 0.80.	
95	48	36	12	0						
Morye × Newland:										
24	I	I	I	I	I	S				
60	I	S	I	I	S	S	S			
52	I	I	I	S	I	S	S			
Plants observed. Plants expected (237:15:3:1).			173	15	2	2		3.488	0.30 and 0.50.	
178	11	2	1							
Billings × Newland:										
24	I	I	I	I	S	S				
47	I	S	I	S	S	S				
Plants observed. Plants expected (3:1:0).			143	42	0			{ .521	{ 0.30 and 0.50.	
139	46	0								
C. I. 438 × Newland:										
16	R-	I	I	R-	S					
Plants observed. Plants expected (48:16:1).			140	51	1			{ 2.244	{ 0.30 and 0.50.	
144	45	3								
C. I. 416-3 × Newland:										
16	I	I	I	I <sup>4</sup> to S-	S					
Plants observed. Plants expected (3:1:0).			142	39	0			{ 1.151	{ 0.20 and 0.30.	
136	45	0								
Rio × Newland:										
1	I	I	I	S						
16	I	I	I	S						
41	I	I	I	S						
Plants observed. Plants expected (1:0).			384	0				{ (?)	{ (?)	
			384	0						

<sup>1</sup> I, immune; R, resistant; R-, moderately resistant; SR, semiresistant; S-, moderately susceptible; S, susceptible.

<sup>2</sup> See table 3.

<sup>3</sup> No segregation to races tested.

<sup>4</sup> Plants classed as immune showed severe necrosis and stunting.

were present in C. I. 416-3 or that environment exerts a considerable influence on the effectiveness of the rust-conditioning factors possessed by this variety.

The rust-conditioning factor in Williston Brown and the factor in Williston Golden allelic to that in Newland are probably identical,

since Williston Brown originated as a brown-seeded selection from a lot of Williston Golden and has been susceptible to all races attacking that variety (3, 6).

It has not been established whether the factors in Bolley Golden and Italia Roma which are allelic to the Newland factor and condition immunity from North American races of rust are identical or allelic. Since the data on the inheritance of rust-conditioning factors in these varieties may be explained by assuming that these factors are identical, they will be so considered until contrary evidence is obtained. Thus, although six differential varieties have factors in the Newland allelomorphic series, the assumption of only four separate factors satisfactorily explains the current data.

Crosses of Newland with three of the differential varieties, Akmolinsk, Abyssinian, and Pale Blue Crimped, were not studied. However, tests indicated that rust reaction in each of these varieties is conditioned by single factors, that of Pale Blue Crimped being allelic to the Ottawa 770B factor and those of Akmolinsk and Abyssinian being linked with the Bombay factor.

#### CROSSES INVOLVING BOMBAY AND PUNJAB

Rust reaction in Bombay and Punjab apparently is conditioned by a common pair of factors as these varieties have given identical reactions to all races with which they have been tested. Bombay and Punjab have been used interchangeably as parents in studying the interaction of the Bombay factor with the rust-resistant factors of other varieties (table 5).

In crosses of Bombay with varieties having single rust-conditioning factors allelic to those in Ottawa 770B (J. W. S., Pale Blue Crimped, and Kenya) or Newland (Williston Brown, Billings, and C. I. 416-3), the  $F_2$  populations segregated for reaction to selected races of *Melampsora lini* in accordance with the simple Mendelian ratios expected if the factors are independently inherited. Likewise, in the crosses of Punjab or Bombay with Buda and Williston Golden, each of which has rust-conditioning factors in both the Ottawa 770B and the Newland series, the observed ratios of segregates satisfactorily approached those expected if all 3 rust-conditioning factors involved in these crosses are independently inherited. Since 1 of the 2 factors conditioning rust reaction in Bolley Golden is allelic to that in Newland, the occurrence of segregates susceptible to race 16 from which both parents were immune indicates that the second rust factor in Bolley Golden is inherited independently of that in Bombay. Insufficient seeds to make a statistically significant analysis for the segregation of rust-conditioning factors were available in the cross Italia Roma  $\times$  Bombay, and the data are inconclusive. However, the assumption that the rust-conditioning factors in this cross are independently inherited gives a poorer fit of the observed to the expected ratio of segregates than does the assumption that 1 of the rust-conditioning factors in Italia Roma is linked with the Bombay factor and that crossing over accounts for the 1 of 48  $F_2$  plants being susceptible to the race attacking neither parent.

Tests on the inheritance of rust reaction in the two progenies of the cross C. I. 438  $\times$  Bombay indicate that the C. I. 438 parent was heterozygous. The ratio of segregates for reaction to race 5 may be ex-

TABLE 5.—*Parental reaction to various races of Melampsora lini and segregation of F<sub>2</sub> plants of crosses involving Bombay or Punjab*

Cross and race tested	Reaction <sup>1</sup> to indicated races of—						χ <sup>2</sup> value	P be- tween—		
	Parent listed first	Parent listed second	F <sub>2</sub> plants							
			R	I	R	S				
Ottawa 770B × Bombay	(2)	(2)			(2)		(2)	(2).		
Bombay × Newland	(3)	(3)			(3)		(3)	(3).		
Williston Brown × Bombay:										
52-----	R	I	I	I	R	S				
55-----	R	S	R	S	R	S				
16-----	S	I	I	I	S	S				
Plants observed			93	35	45	11				
Plants expected (9:3:3:1).			101	34	34	12	4.289	0.30 and 0.30.		
Bombay × Akmolinsk:										
1-----	I	R	I	I	R	S				
5-----	I	S	I	S	R	S				
41-----	S	R	R	S	R	S				
Plants observed			72	41	43	1				
Plants expected (2:1:1:0).			80	40	40	0	1.425	0.20 and 0.50.		
Bombay × J. W. S.:										
1-----	I	I	I	I	I	S				
41-----	S	I	I	S	S	S				
16-----	I	S	I	S	I	S				
Plants observed			117	36	27	12				
Plants expected (9:3:3:1).			108	36	36	12	2.000	0.30 and 0.50.		
Pale Blue Crimped × Bombay:										
7-----	R	I	I	R	S					
Plants observed			99	11						
Plants expected (15:1).			103	7			2.640	0.10 and 0.20.		
Kenya × Bombay:										
6-----	R	I	I	I	I	S				
24-----	R	S	I	S	I	S				
79-----	S	I	I	S	I	S				
Plants observed			31	16	14	3				
Plants expected (9:3:3:1).			36	12	12	4	1.250	0.50 and 0.70.		
Bombay × Abyssinian:										
7-----	I	R	I	R	S					
Plants observed			51	13	0					
Plants expected (3:1:0).			48	16	0		.750	0.30 and 0.50.		
Leona × Bombay:										
6-----	R	I	I	I	R	S				
52-----	S	I	I	S	R	S				
73-----	R	S	I	S	R	S				
Plants observed			85	39	64	2				
Plants expected (2:1:1:0).			94	48	48	0	8.300	0.01 and 0.02.		
Tammes Pale Blue × Punjab:										
2-----	I	I	I	I	I	S				
41-----	I	S	I	I	S	S				
19-----	S	I	I	S	I	S				
Plants observed			114	48	30	0				
Plants expected (2:1:1:0).			96	48	48	0	10.125	(1).		
Punjab × Buda:										
5-----	I	R	I	I	R	S				
3-----	I	SR	I	I	SR	S				
16-----	I	S	I	S	S	S				
41-----	I	SR to S—	S—	S	S—	S				

See footnotes at end of table.

TABLE 5.—*Parental reaction to various races of Melampsora lini and segregation of F<sub>2</sub> plants of crosses involving Bombay or Punjab—Continued*

Cross and race tested	Reaction <sup>1</sup> to indicated races of—							$\chi^2$ value	P be- tween—		
	Parent listed first	Parent listed second	F <sub>2</sub> plants								
			122	31	27	10	2				
Plants observed											
Plants expected (36:12:12:3:1).			108	36	36	9	3	5.103	0.20 and 0.30.		
Bombay × Williston Golden:											
1.....	I	R	I	I	R	S					
2.....	I	S	I	I	S	S					
41.....	S	R	R	S	R	S					
Plants observed											
Plants expected (9:3:3:1).			99	31	45	13		3.772	0.20 and 0.30.		
Bolley Golden × Punjab:											
16.....	I	I	I	I	I	R—	S				
41.....	I	S	I	I	R—	S	R—S				
29.....	S	I	I	S	I	I	S				
Plants observed											
Plants expected (36:12:9:3:3:1).			300	119	70	21	31	9	5.333	0.30 and 0.50.	
Italia Roma × Bombay:											
6.....	I	I	I	I	I	R to S—	S				
24.....	I	S	R to I	I	S	S— to S	S				
105.....	S	I	I	S	I	S	S				
Plants observed											
Plants expected (45:12:3:3:1). <sup>b</sup>			28	12	1	6	1		10.553	0.02 and 0.05.	
Plants expected (11:3:1:1:0). <sup>c</sup>			34	9	2	2	1		6.226	0.10 and 0.20.	
Bombay × Billings:											
1.....	I	I	I	I	I	S					
24.....	I	S	I	S	I	S					
85.....	I	S	I	I	S	S					
Plants observed											
Plants expected (9:3:3:1).			138	30	28	11		9.296	0.02 and 0.05.		
C. I. 438 × Bombay:											
5.....	R	I	I	R to S—	S						
Plants observed											
Plants expected (48:15:1).			130	37	5			3.782	0.10 and 0.20.		
C. I. 438 × Bombay:											
5.....	R	I	I	R to S—	S						
Plants observed											
Plants expected (12:3:1).			134	41	13			1.419	0.30 and 0.50.		
C. I. 416-3 × Bombay:											
16.....	I	I	I	I	S						
24.....	I	S	I	S	S— to S	S					
Plants observed											
Plants expected (48:15:1).			147	40	1			1.908	0.30 and 0.50.		
Rio × Bombay:											
16.....	I	I	I	I	S						
41.....	I	S	I	I	R to S—	S					
19.....	S	I	I	S	I	I	S				
Plants observed											
Plants expected (44:16:3:1:0).			101	77	11	3	0	25.165	(4).		

<sup>a</sup> I, immune; R, resistant; R—, moderately resistant; SR, semiresistant; S—, moderately susceptible; S, susceptible. <sup>b</sup> See table 3. <sup>c</sup> See table 4. <sup>d</sup>  $P < 0.01$ .

<sup>e</sup> Each of 3 pairs of rust-conditioning factors assumed to be independently inherited.

<sup>f</sup> 2 of the 3 pairs of rust-conditioning factors assumed to be allelic.

plained by assuming that in one progeny the C. I. 438 parent contributed two pairs of independently inherited rust-conditioning factors and the Bombay parent one, whereas in the second progeny the assumption that each parent contributed a single pair explains the observed ratio of segregates.

In contrast with the regular segregation and absence of crossing over in hybrids of varieties having factors in the Ottawa 770B or the Newland series, crosses of Bombay or Punjab with varieties having rust-conditioning factors in the same linkage group are marked by irregular ratios and crossing over. Of crosses of varieties having rust-conditioning factors in the Bombay linkage group only the cross Bombay  $\times$  Abyssinian is entirely regular, but only one-third the usual number of plants was tested. The observed ratio of segregates of the cross Bombay  $\times$  Akmolinsk approximates that expected except that 1 of 160 plants was susceptible to race 1, which attacked neither parent. This indicates that the rust-conditioning factors in these varieties are linked rather than allelic. Crossing over was also evident in the cross Leona  $\times$  Bombay, but even if this is disregarded, the observed ratio of segregates deviates significantly from that expected if simple Mendelian inheritance governs the transmission of rust-conditioning factors. In Tammes Pale Blue  $\times$  Punjab no crossing over was observed, but the observed ratio of segregates differs significantly from that expected if the single rust-conditioning factor in each parent is allelic. In the cross Rio  $\times$  Bombay, in which 4 rust-conditioning factors are involved, an insufficient number of plants was studied for a significant statistical analysis, but the deviation of the observed from the theoretical ratio suggests that the Bombay factor for immunity from race 19 is linked with 1 of the 3 Rio factors for immunity from or resistance to race 16. Sufficient F<sub>2</sub> seeds of Bombay  $\times$  Morye were not available for study.

#### CROSSES OF ADDITIONAL DIFFERENTIAL VARIETIES

Some crosses between differential varieties not involving Ottawa 770B, Newland, and Bombay or Punjab (table 6) were studied before it had been determined that most rust-conditioning factors in flax lie in the allelomorphic series or linkage groups represented by the factors in the above-named varieties. Other crosses were studied later to verify the allelic or linkage relations as determined in the tests of hybrids involving Ottawa 770B, Newland, and Bombay and to demonstrate the effectiveness of selected races of the pathogen in resolving the F<sub>2</sub> populations into their phenotypic components.

Without exception the data (table 6) substantiate the conclusions regarding interaction of the rust-conditioning factors in the differential varieties based on the study of crosses with Ottawa 770B, Newland, and Bombay or Punjab. No F<sub>2</sub> plant susceptible to races attacking neither parent was observed in the crosses Williston Golden  $\times$  J. W. S., Morye  $\times$  J. W. S., Rio  $\times$  J. W. S., and Pale Blue Crimped  $\times$  Buda, hybrids in which each parent has a rust-conditioning factor allelic to that in Ottawa 770B. Likewise, in the crosses Williston Brown  $\times$  Williston Golden and Bolley Golden  $\times$  Williston Golden, varieties having a factor for rust reaction allelic to that in Newland, and in the cross Akmolinsk  $\times$  Abyssinian, each of which has a rust-conditioning factor linked with that in Bombay, no F<sub>2</sub> segregate sus-

TABLE 6.—*Parental reaction to various races of Melampsora lini and segregation of F<sub>2</sub> plants of crosses not involving Ottawa 770B, Newland, and Bombay or Punjab*

Cross and race tested	Reaction <sup>1</sup> to indicated race of—								χ <sup>2</sup> value	P be- tween—		
	Parent listed first	Parent listed second	F <sub>2</sub> plants									
			R	I	I	I	R	S				
Williston Brown × J. W. S.:			R. S.	I S	I R	I I	R S	S S				
52-----												
7-----												
24-----												
Plants observed					116	33	32	11				
Plants expected					108	36	36	12				
(9:3:3:1).									1.369	0.50 and 0.70.		
Pale Blue Crimped × Williston Brown:			R—	R	R	S						
7-----												
Plants observed					120	8						
Plants expected					120	8						
(15:1).									0.000	(3).		
Abyssinian × Williston Brown:			R	R	R	S						
7-----												
Plants observed					58	6						
Plants expected					60	4						
(15:1).									1.067	0.30 and 0.50.		
Williston Brown × Williston Golden:			R	R	R	S						
7-----												
52-----												
Plants observed					64	0						
Plants expected					64	0						
(1:0).									(3)	(3).		
Akmolinsk × J. W. S.:			R S	I I	I I	I S	R S	S S				
1-----												
5-----												
9-----												
Plants observed					112	32	40	8				
Plants expected					108	36	36	12				
(9:3:3:1).									2.369	0.30 and 0.50.		
Akmolinsk × Abyssinian:			R S	I R	I R	R	S	S				
1-----												
3-----												
Plants observed					130	38	0					
Plants expected					126	42	0					
(3:1:0).									.508	0.30 and 0.50.		
Buda × Akmolinsk:			I SR	S S	I and R SR	I and R SR	I S	I S	S S			
7-----												
3-----												
9-----												
4-----												
Plants observed					63	24	13	5	3	1		
Plants expected					62	20	15	5	5	2		
(36:12:9:3:3:1).									1.984	0.80 and 0.90.		
Williston Golden × Akmolinsk:			R R S R	R S R S	R R S R	R S R S	R R S S	R S S S				
1-----												
7-----												
2-----												
5-----												
Plants observed					113	32	27	13	5	2		
Plants expected					108	36	27	9	9	3		
(36:12:9:3:3:1).									4.341	0.50 and 0.70.		

See footnotes at end of table.

TABLE 6.—*Parental reaction to various races of *Melampsora lini* and segregation of F<sub>2</sub> plants of crosses not involving Ottawa 770B, Newland, and Bombay or Punjab—Continued*

Cross and race tested	Reaction <sup>1</sup> to indicated race of—					$\chi^2$ value	P be- tween—
	Parent listed first	Parent listed second	F <sub>2</sub> plants				
Williston Golden × J. W. S.: 1----- 7----- 2-----	R R S	I S I	I R I	R R S	I S I S		
Plants observed.				138	39	15 0	
Plants expected (11:4:1:0).				132	48	12 0	2.710 0.20 and 0.30.
J. W. S. × Bolley Golden: 24----- 21----- 19-----	I S I	I I S	I I I	I R I	I S I S		
Plants observed.				321	85	69 22 22 8	
Plants expected (39:12:9:3:3:1).				296	99	74 25 25 8	4.922 0.34 and 0.50.
Morye × J. W. S.: 16----- 24-----	I I	S I	I I	S I	S S		
Plants observed.				190	2	0	
Plants expected (63:1:0).				189	3	0	.338 0.50 and 0.70.
Rio × J. W. S.: 7----- 16----- 20-----	I I S	S S I	I I I	I R S	S S I		
Plants observed.				104	54	49 3 0	
Plants expected (32:16:15:1:0).				105	53	49 3 0	.081 0.99 and 1.00.
Pale Blue Crimped × Buda: 7-----	R—	I	I	R	S		
Plants observed.				91	25	0	
Plants expected (3:1:0).				87	29	0	.736 0.30 and 0.50.
Williston Golden × Abyssinian: 47----- 52----- 16----- 68-----	R R R S	I to R S S I to R	R R R R	R R S R	R R S R	'S S S S	
Plants observed.				72	19	21 21 12 6 5 5	
Plants expected (27:9:9:9:3:3:3:1).				69	23	23 23 8 8 8 3	6.946 0.30 and 0.50.
Bolley Golden × Williston Golden: 7----- 16----- 52----- 24----- 73-----	I I I I R	R R R S S	I I I I R	I R R R S	I R R S S	S S S S S	
Plants observed.				104	39	31 9 5 0	
Plants expected (36:12:12:3:1:0).				103	35	35 9 3 0	2.392 0.50 and 0.70.

<sup>1</sup> I, immune; R, resistant; R—, moderately resistant; SR, semiresistant; S—, moderately susceptible; S<sub>s</sub>, susceptible.

<sup>2</sup> P, 1.00.

<sup>3</sup> No segregation to races tested.

ceptible to races attacking neither parent was observed. Conversely, in the crosses Williston Brown × J. W. S., Pale Blue Crimped × Williston Brown, Abyssinian × Williston Brown, Akmolinsk ×

J. W. S., Buda  $\times$  Akmolinsk, Williston Golden  $\times$  Akmolinsk, J. W. S.  $\times$  Bolley Golden, and Williston Golden  $\times$  Abyssinian, varieties in which the factors for rust reaction are not allelic or linked, the F<sub>2</sub> populations segregated for reaction to rust into ratios approximating those expected if the factors conditioning rust reaction are independently inherited.

Of special interest is the test of the cross Williston Golden  $\times$  Abyssinian. Rust reaction in the former is conditioned by two factors, one allelic to that in Ottawa 770B and the other allelic to that in Newland. The single rust-conditioning factor in Abyssinian lies in the Bombay linkage group. By inoculating the F<sub>2</sub> plants successively with races 47, 52, 16, and 68, the eight phenotypic groups theoretically possible in such a trifactorial hybrid were differentiated and  $\chi^2$  for fit of the observed to the theoretical 27:9:9:9:3:3:3:1 ratio of segregates gave a P value lying between 0.30 and 0.50, indicating good agreement between the data and the hypothesis.

#### DISCUSSION AND CONCLUSIONS

Suggested genotypes of the 16 rust-differential flax varieties are given in table 7. These 16 varieties possess 22 pairs of rust-conditioning factors of which at least 19 are distinct. Other varieties have additional rust-conditioning factors distinct from those possessed by the 16 differentials.

Seven of the differential varieties have rust-conditioning factors in the LL series, 6 in the MM series, and 5 in the NN series. Each factor in the LL series appears to be distinct. Of the varieties having factors in the MM series Williston Brown and Williston Golden and Bolley Golden and Italia Roma appear to have common factors. The study on the inheritance of pathogenicity in flax rust (6) indicates that one of the unplaced factors in Morye may be identical with the

TABLE 7.—*Suggested genotypes of rust-differentiating and other varieties of flax*

Variety	C. I. No.	Pairs of factors for rust reaction	Suggested genotype
<i>Differential varieties:</i>			
Ottawa 770B	355	1	LL mm nn.
Newland	188	1	ll MM nn.
Bombay	42	1	ll mm NN.
Williston Brown	803-1	1	ll M <sup>1</sup> M <sup>1</sup> nn.
Akmolinsk	515-1	1	ll mm N <sup>1</sup> N <sup>1</sup> .
J. W. S.	708-1	1	L <sup>2</sup> L <sup>2</sup> mm nn.
Pale Blue Crimped	647	1	L <sup>2</sup> L <sup>3</sup> mm nn.
Kenya	709-1	1	L <sup>4</sup> L <sup>4</sup> mm nn.
Abyssinian	701	1	ll mm N <sup>2</sup> N <sup>2</sup> .
Leona	836	1	ll mm N <sup>3</sup> N <sup>3</sup> .
Tammes Pale Blue	333-1	1	ll mm N <sup>4</sup> N <sup>4</sup> .
Buda	270-1	2	L <sup>1</sup> L <sup>1</sup> M <sup>2</sup> M <sup>2</sup> nn.
Williston Golden	25-1	2	L <sup>3</sup> L <sup>5</sup> M <sup>1</sup> M <sup>1</sup> nn.
Bolley Golden	644	2	ll M <sup>3</sup> M <sup>3</sup> nn.
Italia Roma	1005-2	2	ll M <sup>2</sup> M <sup>2</sup> (?) .
Morye <sup>1</sup>	112	3	L <sup>0</sup> L <sup>6</sup> mm (?) .
<i>Other varieties:</i>			
Billings	184	1	ll M <sup>4</sup> M <sup>4</sup> nn.
Minn. 25-107 <sup>4</sup>	438	1 and 2	L <sup>5</sup> L <sup>7</sup> mm nn.
Pale Verbena <sup>4</sup>	416-3	1 and 2	M <sup>0</sup> M <sup>5</sup> nn.
Rio	280	3	L <sup>0</sup> L <sup>6</sup> M <sup>6</sup> M <sup>6</sup> N <sup>5</sup> N <sup>5</sup> .

<sup>1</sup> This variety has 1 factor lying outside the L, M, or N series.

<sup>2</sup> The location of this factor not definitely established but probably in the N series.

<sup>3</sup> Not tested.

<sup>4</sup> Not pure; some plants have 1 and others 2 resistant factors.

factors in the *NV* series conditioning rust reaction in Tammes Pale Blue. As there was no crossing over in hybrids between varieties having factors in either the *LL* series or the *MM* series, these factors are considered to be allelomorphic. Crossing over and irregular segregation occurred in most hybrids involving varieties having factors in the *NV* series; therefore these factors are considered to be linked rather than allelomorphic.

Studies on the inheritance of pathogenicity in hybrids between races of flax rust (5, 6) have shown definite linkage between factors for virulence on certain varieties. All  $F_2$  cultures of hybrids between races 22 and 24 that attacked Kenya ( $L^4L^4$ ) also attacked Pale Blue Crimped ( $L^3L^3$ ). Since Kenya is highly resistant to some races to which Pale Blue Crimped is moderately susceptible, the single pairs of rust-conditioning factors in these varieties are probably distinct. The factors for reaction to rust in Newland and Billings are allelomorphic, and factors for virulence on these varieties are linked. Virulence on Akmolinsk ( $N^1N^1$ ), Abyssinian ( $N^2N^2$ ), and Leona ( $N^3N^3$ ) also was inherited as a unit in the hybrids between races 22 and 24. The single pair of rust-conditioning factors in each of these varieties appears to be distinct, as Akmolinsk is susceptible to several races to which Abyssinian and Leona are resistant and Leona is resistant to some races that attack both Akmolinsk and Abyssinian. The distinctive character of the factors for rust reaction is further emphasized by pathogenicity tests of  $F_2$  cultures of race 6  $\times$  race 22 (hybrid A) (6). In this cross both parental gametes possessed a factor for virulence on Akmolinsk, but only the race 22 gamete possessed factors for virulence on Abyssinian and Leona. Each of the 74  $F_2$  cultures studied was virulent on Akmolinsk, but only 17 on Abyssinian and Leona. In the case of Kenya and Pale Blue Crimped, Newland and Billings, and Akmolinsk, Abyssinian, and Leona there was a parallelism of linkage between factors for virulence in the pathogen and those for rust reaction in the host. However, the linkage between factors for pathogenicity in the fungus extended beyond the linkage groups in the host. Thus, in a hybrid of a race 6 gamete (avirulent on Buda, virulent on Akmolinsk) with a race 24 gamete (virulent on Buda, avirulent on Akmolinsk) none of the 96 cultures studied was virulent on both Buda and Akmolinsk (5). In hybrids between race 22, to which Buda was highly susceptible, and races 6 and 24, to which Buda was resistant and moderately susceptible, respectively, all cultures to which Buda was highly susceptible had the race 22 factors for virulence on Akmolinsk, Abyssinian, and Leona (6). The Buda factors for rust reaction are in the *LL* and *MM* allelomorphic series and those of Akmolinsk, Abyssinian, and Leona in the *NV* linkage group.

A knowledge of the intraaction and interaction of factors for rust reaction in the host and those for pathogenicity in the pathogen should assist in programs to develop rust-resistant varieties of crop plants by indicating the potential pathogenicity of new races and by serving as a guide in the selection of parental material by indicating the possibility of incorporating specific rust-conditioning factors into the progeny.

The studies on the genetics of pathogenicity in *Melampsora lini* (6) give little indication that there are inherent limitations to the pathogenic capacity of races developed through hybridization of existing races. Virulence on all varieties except those possessing the Wil-

liston Brown  $M^1M^1$  factor was inherited as a recessive character. Race 22 attacks all varieties with which it has been tested except those having the J. W. S.  $L^2L^2$  and the Bombay  $NN$  factors. Consequently, it already possesses the homozygous recessives for virulence on all of the varieties having known linkage relations which might impede the accumulation of factors for virulence. The factors of race 24 for virulence on Bombay are inherited independently of all factors for virulence possessed by race 22 including those on Akmolinsk, Abyssinian, Leona, and Tammes Pale Blue, varieties having rust-conditioning factors in the Bombay  $NV$  linkage group. The inheritance of factors for virulence on J. W. S. has not been determined, but Vallega (11) reported the isolation in Argentina of a race of *M. lini* attacking J. W. S. in addition to the varieties attacked by race 22. Thus, resistance to all known races and to those that have been proved to be theoretically possible can be obtained only by combining the J. W. S. and Bombay factors for rust reaction. The possibility of combining in one race factors for virulence on J. W. S. and Bombay has not been explored, but the studies thus far completed indicate no impediment to the occurrence of such a race.

At least 19 distinct factors condition reaction of the 16 rust-differentiating varieties of flax. All but 8 of these condition immunity from or resistance to the races of *Melampsora lini* that have been isolated from North American rust collections. Consequently, there is an abundance of parental material resistant to North American races. The problem would become complicated if races having the pathogenic capacities of those obtained from South America should become established. Races attacking Bombay, the only variety known to be immune from all South American races, occur in North America. In the flax-growing region of the Midwestern States, sexual reproduction and probably hybridization precede the initiation of rust infection each year. This would afford an opportunity for the development of races having almost infinitely varied pathogenic capacities.

The fact that sexual reproduction precedes infection in the mid-western flax-growing region may have some ameliorating consequences. Virulence is predominantly recessive so that hybrid races combine the avirulence of their parents. This may account for the predominance in the Midwest of races having a restricted varietal host range (3). All collections obtained from Oregon, Argentina, Brazil, and Uruguay have carried races attacking several or most of the differential varieties. In these regions rust may persist throughout the year in the uredinal stage, permitting the perpetuation of the more vigorous races having the widest host range.

At the present time, any one of 15 or more factors satisfactorily conditions resistance to North American races. However, because of the possibility of the development or introduction of new races the feasibility of incorporating multiple resistance factors into new varieties should be considered. By proper selection of parents from varieties listed in table 7, it should be possible to obtain lines having 3 or 4 pairs of rust-conditioning factors, 1 each from the  $LL$ ,  $MM$ , and  $NV$  series and 1 of the unplaced factors from Bolley Golden or Morye. From the data presented in this paper it appears unlikely that more than 1 pair of the  $LL$  or  $MM$  factors can be incorporated into a line. Since evidence of crossing over occurred in the  $NV$  series, it may be possible to obtain a doubling up of the factors in this series.

Although the occurrence of rust-conditioning factors in allelomorphic series limits the number of factors that may be incorporated into a variety, a knowledge of the interaction of these factors may facilitate a breeding program. Since resistance to rust invariably has been inherited as a dominant character, all the progeny of crosses of varieties having allelomorphic factors for resistance to races with which the investigator must contend should be resistant to these races. In crosses between parents having allelomorphic factors, all hybrids susceptible to a race attacking one parent but not the other should be homozygous for factors conditioning resistance to races having the reverse reaction. For example, the progeny of Newland ( $MM$ )  $\times$  Bolley Golden ( $M^3M^3$ ), both of which are immune from all races found in the Midwest, should be immune in that region. In this same cross all  $F_2$  plants susceptible to race 58, to which Bolley Golden is susceptible and Newland immune, should be homozygous for the Bolley Golden  $M^3M^3$  factors. Conversely, all  $F_2$  plants susceptible to race 47, to which Newland is susceptible and Bolley Golden immune, should be homozygous for the Newland  $MM$  factors. Thus, it is possible, by testing the reaction of  $F_2$  plants of hybrids having allelomorphic factors with appropriate races, to eliminate all plants not homozygous for the desired rust-conditioning factors. This permits discarding approximately three-fourths of the material before it reaches the  $F_3$  generation.

#### SUMMARY

The interaction of factors conditioning reaction to rust in 20 flax varieties, including the 16 that have served as differentials for the identification of physiologic races of flax rust (*Melampsora lini*), was studied by determining the reaction of  $F_2$  populations.

By the use of selected races of the pathogen it was demonstrated that at least 19 pairs of factors are involved in conditioning the reaction to rust of the 16 differential varieties. Of the 19 pairs of factors 16 lie in 3 series, or linkage groups: 7 in the Ottawa 770B, or  $LL$ , series; 4 in the Newland, or  $MM$ , series; and 5 in the Bombay, or  $NN$ , series. There was no indication of crossing over between factors in the  $LL$  or  $MM$  series, and factors in each of these series are considered to be allelomorphic. Some crossing over and irregular segregation ratios were obtained in hybrids between varieties having rust-conditioning factors in the  $NN$  series, and these are considered to be linked rather than allelomorphic. Additional rust-conditioning factors were found in the 4 other varieties studied.

Varieties in which single pairs of factors in the  $LL$  series condition reaction to rust are Ottawa 770B, J. W. S., Pale Blue Crimped, and Kenya. One of the two pairs of rust-conditioning factors in Buda, Williston Golden, and C. I. 438 lies in the  $LL$  series as does one of the three pairs of factors in Morye.

Single pairs of factors in the  $MM$  series condition reaction to rust in Newland, Williston Brown, and Billings. One of the two rust-conditioning factors in Buda, Williston Golden, Bolley Golden, Italia Roma, and C. I. 416-3 lies in the  $MM$  allelomorphic series. Probably the  $MM$  factors in Williston Brown and Williston Golden are identical, and those in Bolley Golden and Italia Roma may also be.

Single pairs of factors in the  $NN$  linkage group condition reaction to rust in Bombay, Akmolinsk, Abyssinian, Leona, and Tammes Pale

Blue, Bombay and Punjab appear to have identical factors. One of the two pairs of rust-conditioning factors in Italia Roma may lie in the *NN* linkage group.

One pair of rust-conditioning factors in Bolley Golden and two pairs in Morye have not been placed. Hybrids between the latter and varieties with factors in the *NN* group were not studied.

Three pairs of factors condition reaction to rust in Rio, one pair apparently lying in each of the allelomorphic series or linkage groups.

There is a parallelism in linkage between certain factors for rust reaction in the host and those for pathogenicity in the fungus. Virulence on Pale Blue Crimped ( $L^3L^3$ ) and Kenya ( $L^4L^4$ ), varieties having allelomorphic factors, was inherited as a unit, as was virulence on Akmolinsk ( $N^1N^1$ ), Abyssinian ( $N^2N^2$ ), and Leona ( $N^3N^3$ ). However, pathogenicity to these three varieties having factors in the *NN* series was also linked with that to Buda ( $L^1L^1M^2M^2$ ), indicating that linkage between pathogenic factors transcends the linkage groups conditioning resistance in the host.

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## EMBRYOLOGY OF PECAN<sup>1</sup>

By J. W. MCKAY

*Associate cytologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture*

### INTRODUCTION

Part of the fruits of a pecan tree frequently drop at some stage of development between pollination and maturity. The formation of the embryo, usually referred to as filling, also is sometimes faulty and incomplete. Several studies have been made on the development of the nut of the pecan (*Carya illinoensis* (Wang.) K. Koch), but investigators have not agreed on the identity and rate of development of certain embryological structures. In order to study effectively these problems it is necessary to identify the embryological tissues in the nut and to know the rate and method of their development. Without such information it is difficult, for example, to determine the physiological processes involved in the transfer of elaborated food into the kernel.

The purpose of the present investigation was (1) to trace the morphological changes that take place in the pecan nut from the time of pollination to maturity and (2) to clarify the relations between the endosperm and the embryo. This paper deals primarily with the later stages of nut development during which the embryo is formed.

Woodroof and Woodroof (13)<sup>2</sup> and Woodroof (15) described the early embryology of the pecan, but these writers did not agree with Shuhart (10) as to the time of fertilization or the beginning of embryo growth. Langdon (6) described the development of the ovule and fruit structures of *Carya glabra* (Mill.) Sweet, a related species of hickory. The embryology of *Juglans regia* L., another important species of the Juglandaceae, reported by Nast (7, 8), is similar in many details to that of pecan although the chronological order of development is quite different. Finch and Van Horn (5) gave a complete account of the later stages of nut filling in the pecan and discussed the physiological conditions of the tree that are favorable to the production of well-filled nuts.

### MATERIALS AND METHODS

Pistillate flowers and fruits of the Greenriver variety of pecan were collected at Beltsville, Md., at weekly intervals from May to November. The Greenriver pecan is one of the northern varieties that originated in southern Indiana. Because of its origin the total develop-

<sup>1</sup> Received for publication December 14, 1945.

<sup>2</sup> Italic numbers in parentheses refer to Literature Cited, p. 283.

mental period of its nuts is about a month less than that of nuts of southern varieties; during average seasons this variety produces fairly well-filled nuts in Maryland. In addition, young fruits of the Schley variety from the time of blossoming to 49 days after pollination were available from Albany, Ga., for study.<sup>3</sup>

After the extraneous ovarian tissue had been removed, ovules in early stages of development were fixed in Langlet's modification of Na-vashin's solution. Large fruits were fixed in formalin-aceto-alcohol; they were more easily dissected after fixation than when fresh, and fixation of tissue was satisfactory for studying gross changes in the endosperm and embryo. Tissue was dehydrated in the ethyl-butyl-alcohol series, embedded in paraffin, cut 10 $\mu$  to 15 $\mu$  thick, and stained in iron-alum haematoxylin or safranine and fast green.

Photomicrographs of young ovule sections were made with a Zeiss Miflex camera, 9  $\times$  ocular and 8- and 4-mm. objectives. Sections of the large ovules were photographed with a Bausch & Lomb H camera with Micro Tessar lenses. Mature fruits were sectioned by hand with a sharp knife and photographed on a ground-glass surface with illumination from below to avoid shadows. Some of the dissected ovules were stained lightly in aqueous safranine and photographed in water, as the delicate endosperm at certain stages is formless if removed from a supporting liquid.

Some fruits on a particular tree were as much as a week or 10 days later in endosperm development than others on the same tree; it was therefore necessary to use the average of a number of fruits collected on any one date as representative of that weekly stage of development. The same procedure was also necessary in regard to weekly stages of embryo growth.

#### POLLINATION

Pollination occurs about June 1 in Maryland, the exact time depending upon the season and variety. At the time of pollination a fully formed embryo sac is present in the nucellus of the pecan ovule (fig. 1, A). Pistillate flowers of the variety Schley grown at Albany, Ga., also contain mature embryo sacs at the time of stigma receptivity. The method of embryo-sac formation is similar to that described by Woodroof (15) except that all of the stages appear to be completed prior to stigma receptivity. Four megasporae are formed by the megasporae mother cell about 10 days before pollination, and during this 10-day period the chalazal megasporae through three successive nuclear divisions forms the normal angiosperm type of embryo sac as described by Sharp (9). Woodroof (15) found that four megasporae were present in the nucellus at the time of stigma receptivity and the embryo sac matured a week later. Shuhart (10) reported an eight-nucleate megagametophyte in pecan at the time of pollination; fusion of the two polar nuclei occurred immediately after the eight nuclei of the embryo sac were fully differentiated.

The course of pollen tubes through the tissue of the stigma, style, and ovary was difficult to follow with the fixatives and stains used. It was not possible to determine whether pollen tubes normally enter

<sup>3</sup> Acknowledgement is due Max B. Hardy, U. S. Department of Agriculture Pecan Laboratory, Albany, Ga., for supplying this material.

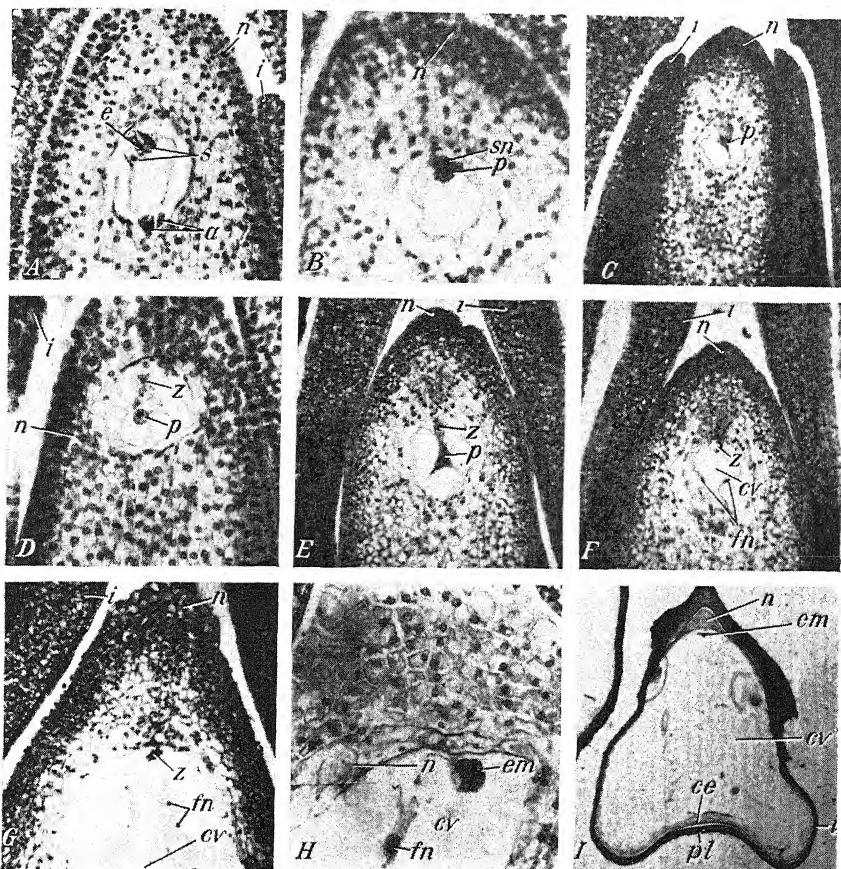


FIGURE 1.—Longitudinal sections of ovules of Greenriver and Schley pecans, showing various stages of development. *A*, Mature embryo sac at time of pollination, June 1. *B*, Embryo sac 4 days after pollination, showing primary endosperm nucleus and union of egg and sperm, or syngamy. *C*, Primary endosperm nucleus and slightly out-of-focus zygote 4 days after pollination. *D*, Primary endosperm nucleus and zygote 6 days after pollination. *E*, Primary endosperm nucleus and zygote with single integument enclosing the nucellus 10 days after pollination. *F*, Dormant zygote with endosperm beginning to develop. *G*, Endosperm more extensively developed than in *F* and dormant zygote 42 days after pollination. Collapse of the central vacuole due to fixation caused shrinkage of the endosperm from a peripheral position in the embryo-sac cavity. *H*, Embryo of approximately eight cells 48 days after pollination, July 19. *I*, Small, undifferentiated embryo and greatly expanded endosperm 62 days after pollination, August 2. The central vacuole occupied most of the ovule, and cellular endosperm appeared adjacent to the seed coat. The nucellus was reduced to a small cap of tissue at the micropylar end of the ovule. *A, D, H*,  $\times 174$ ; *B*,  $\times 186$ ; *C, E, F, G*,  $\times 90$ ; *I*,  $\times 10$ . *a*, Antipodal; *ce*, cellular endosperm; *cv*, central vacuole of the endosperm; *e*, egg; *em*, embryo; *fn*, free nuclei of the endosperm; *i*, integument; *n*, nucellus; *p*, primary endosperm nucleus; *pl*, placenta; *s*, synergids; *sn*, syngamy; *z*, zygote.

the ovule through the micropyle or the chalaza, although they were usually recognizable after they entered the cavity of the embryo sac.

The ovule is orthotropic and sessile and has a single integument that encloses the nucellus in 4 to 10 days after pollination (fig. 1, *C*

and *E*). The integument later becomes the thin, brown, membranous seed coat, or outer covering of the kernel.<sup>4</sup>

### FERTILIZATION

Union of the polar-fusion nucleus with one of the gametes from the pollen tube takes place about 4 days after pollination (fig. 1, *C*). The large primary endosperm nucleus thus formed is the most prominent feature of the embryo sac during the 14-day period after pollination (fig. 1, *B-E*). It lies embedded in a thick strand of cytoplasm near the center of the sac, with large vacuoles on either side.

Union of the second male gamete and the egg occurs 4 to 7 days after pollination (fig. 1, *B*). Long-delayed fertilization of the egg as reported by Woodroof and Woodroof (13) was not observed. The interval between fertilization of the egg and that of the polar-fusion nucleus by the two male gametes may be a matter of hours, as both fusions were observed in ovules fixed 4 days after pollination (fig. 1, *B, C*). Langdon (6) stated that in *Carya glabra* the polar-fusion nucleus united with the male gamete only a few hours before fusion of the egg and the second male gamete. Woodroof (15) reported union of the polar-fusion nucleus and the male gamete in *C. illinoensis* 14 days after pollination and fertilization of the egg probably 35 to 42 days after pollination. The present studies show that for 7 to 14 days after the egg is fertilized the synergids remain as dark-staining, irregularly shaped cells.

The zygote remains inactive at the micropylar end of the embryo sac about 42 days after pollination (fig. 1, *G*). The length of this inactive period varies somewhat, depending upon the time the pistillate flower is pollinated and other factors, as some ovules collected 49 days after hand-pollination showed the zygote still in a resting stage. In none of the ovules examined, however, was the inactive period less than 42 days.

### GROWTH PERIODS OF THE FRUIT

The development of the pecan fruit subsequent to the first 14 days after pollination may be divided into two approximately equal periods: (1) Endosperm development, which requires about 77 days; and (2) embryo growth, or filling, which requires about 70 days. This division of the total growth period on the basis of morphological features corresponds almost exactly to the division made by Crane and Hardy (3) on the basis of growth behavior. These writers reported two periods in the development of the pecan fruit: (1) The period of growth in size, which begins at blossoming and probably ends with hardening of the shell, at which time the nuts have attained full size; and (2), the period of filling of the nut, which probably begins about the time the shell begins to harden and ends with separation of the nut from the shuck. In the present investigation it was found that the stage of maximum endosperm development occurs just as the shell begins to harden at the blossom end of the fruit and the embryo reaches macroscopic size. Thus, the beginning of shell hardening

<sup>4</sup> Since the term "kernel" is widely used to indicate the edible portion of the pecan nut, it should be understood that it includes at maturity the seed coat, embryo, and remains of the endosperm.

coincides with maximum endosperm development and the beginning of rapid embryo growth, or filling.

The different growth periods of fruit and seed are the most striking features of pecan-fruit development. The fruit enlarges during the first 56 days after pollination to about half its full size before there is macroscopic evidence of ovule enlargement. During the third month after pollination ovule enlargement is accelerated, and at the end of this period both ovule and fruit are approximately full-sized. Filling of the nut, which means rapid enlargement of the embryo, begins about the time the fruit and ovule attain maximum size.

#### ENDOSPERM DEVELOPMENT

The primary endosperm nucleus starts dividing about 14 days after pollination, or about the middle of June in Maryland (fig. 1, *F*). A rapid increase is noted in the size of the embryo-sac cavity and growth of the endosperm 28 days after initiation of endosperm growth, 42 days after pollination (fig. 1, *G*). From the beginning the most striking feature of the endosperm is the large central vacuole filled with liquid. During the early stages of ovule growth the free nuclei of the endosperm are embedded in a strand of cytoplasm along the outer margin of the central vacuole and in contact with the nucellus. The layer of endosperm tissue in figure 1, *G*, was withdrawn from the margin of the cavity by the fixative.

Growth of the endosperm is free-nucleate around the outer margin of the central vacuole for about 42 days, or until approximately August 1, about 60 days after pollination. At this time cell walls are formed beginning at the chalazal end of the ovule just above the placenta and progressing along the outer margin of the central vacuole to the micropylar end. The ovule is 5 to 7 mm. long at this time (fig. 2, *A, i*). A longitudinal section of an ovule collected on August 2 shows a thin layer of cellular endosperm around the outer margin of the central vacuole and adjacent to the seed coat (fig. 1, *I*). At this stage the nucellus except for a small cap of tissue at the micropylar end has been absorbed by the rapidly expanding endosperm.

The progressive development of the ovule, particularly the endosperm and the embryo, throughout the season is shown in chronological order in figures 2 and 3. Enlargement of the ovule is very rapid during the latter part of July and early August (fig. 2, *A, h-k*). This is also the period of most rapid increase in fruit size. Growth of the ovule during this period is accompanied by a striking enlargement of the central vacuole, and there is also some increase of cellular endosperm tissue on the inner side of the seed coat. Under normal conditions the ovule is very turgid during this period because of its watery contents; the pressure developed by the central vacuole is probably the force that pushes the elongating ovule into the lower part of the nut, as suggested by Woodroof and Woodroof (13). This force is so great that it is practically impossible to dissect fresh fruits during this period without breaking the seed coat and endosperm tissue and allowing the vacuole contents to escape and the ovule to collapse. Fruits preserved in formalin-aceto-alcohol are much easier to dissect, as the fixative destroys the turgidity of the central vacuole.

At the time shell hardening begins, about the last week in August, the fruit is almost full-sized (fig. 2, *B, d*) and the central watery vacu-

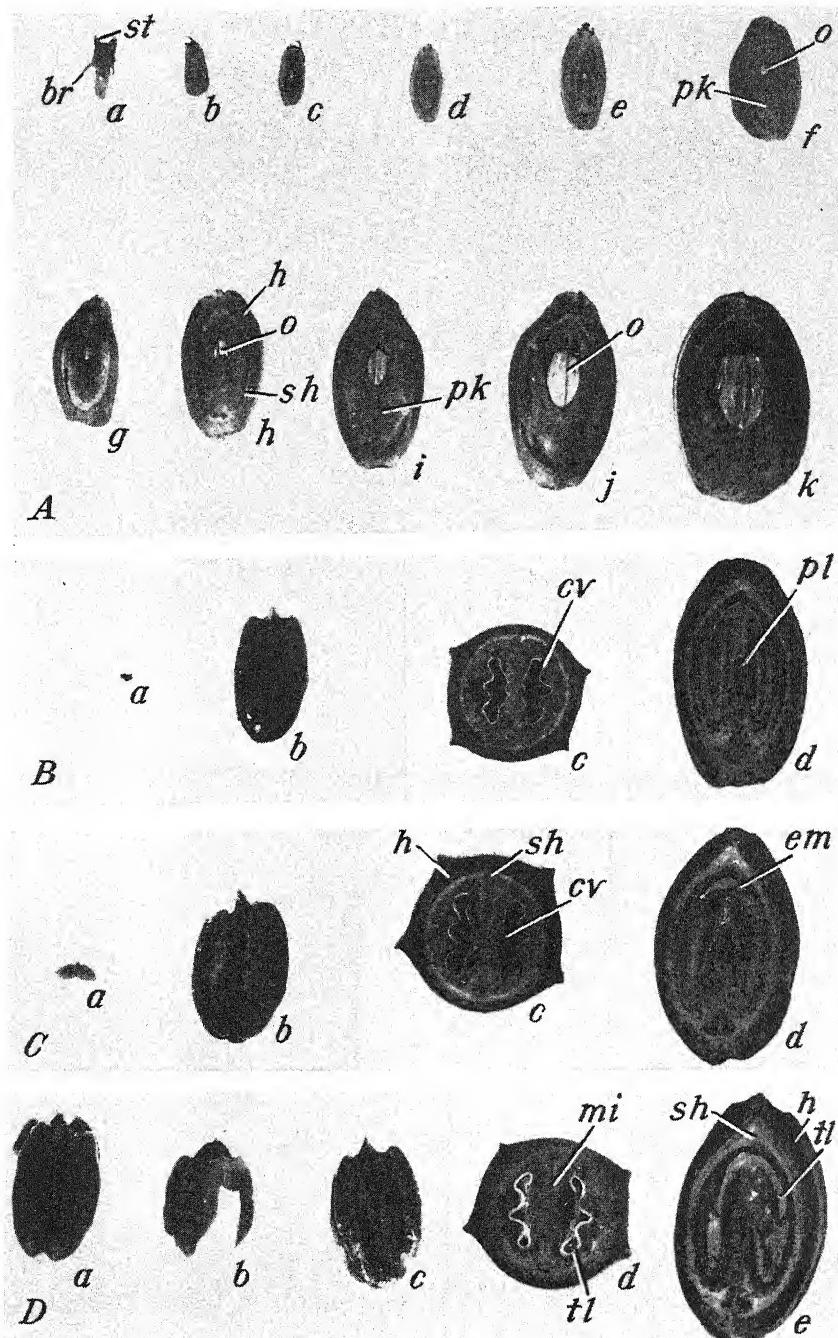


FIGURE 2.—For explanatory legend see opposite page.

ole of the endosperm has reached its greatest proportion. At this stage the ovule is more than two-thirds of its full size and is very turgid because of its liquid contents. Thor and Smith (12, p. 109) referred to this stage as "the kernel in the watery stage." The embryo, which first becomes macroscopic at this time, may be observed as a small bit of solid white tissue about 1 to 2 mm. long in the micropylar tip of the ovule (fig. 2, *B, a, d*). Since the embryo enlarges rapidly from this point on and the endosperm has reached its maximum development, the beginning of shell hardening is a convenient marker to separate approximately the two main phases of seed formation, namely, endosperm development and embryo growth, or filling. The period of growth in size of the nut as reported by Crane and Hardy (3) is then correlated with endosperm development, and their period of filling of the nut is correlated with embryo growth.

Beginning about the first week in September, as the ovule attains full size, a gelatinous layer of cellular endosperm is formed on the inner wall of the seed coat. However, in fruits collected on September 2 this membranous layer was still too fragile to be dissected from the ovule (fig. 2, *C, c, d*). In fruits collected on September 8 the peripheral cellular portion of the endosperm was sufficiently well developed to permit dissection from the ovule in fixed material. In figure 2, *D, c*, is shown the seed coat of an ovule from which the endosperm (*a*) and embryo (*b*) were dissected. The central vacuole of the endosperm is greatly reduced at this stage, as may be observed by comparing figure 2, *D, d*, with *C, c*. This reduction is due to encroachment of the embryo and a rapid increase in the cellular portion of the endosperm. The cellular endosperm at this stage is transparent and very homogeneous in structure. In order to photograph the tissue it was necessary to stain it lightly in 1-percent aqueous safranine and to submerge it in water so that it would retain its shape. In figure 2 it may be noted that the cellular endosperm (*D, a*) conforms to the shape of the ovule (*D, c*), indicating the peripheral position of the former within the seed coat. The endosperm apparently at this stage of development was described by Woodroof and Woodroof (13, p. 1057) as a "thick succulent pad of material" included within the folds of the cotyledons, the origin and purpose of which "has not been determined."

From this time on the endosperm is rapidly surrounded by the folds of the cotyledons and within 14 days—by September 22—was absorbed and reduced to a thin membrane (fig. 3, *C, a; D, a; E, a*; and fig. 4, *B*).

FIGURE 2.—Pecan-fruit structures showing chronological order of development;  $\times$  approximately 1. *A*, Longitudinal sections of fruits in plane of the middle septum showing approximately weekly stages of growth and increase in size of fruit and ovule, beginning 6 days after pollination (*a*) and ending 77 days after pollination, August 17 (*k*). *B*, Dissected fruits collected August 25: *a*, Embryo; *b*, seed coat; *c*, cross section showing central vacuole of the ovule; *d*, longitudinal section perpendicular to middle septum showing placenta and ovule two-thirds its full length. *C*, Dissected fruits collected September 2: *a-d*, Same as in *B*. *D*, Dissected fruits collected September 8: *a*, Endosperm dissected from within folds of the cotyledons and slightly stained in 1-percent aqueous safranine; *b*, embryo with cotyledons slightly more than half as long as the ovule; *c*, seed coat; *d*, cross section showing white solid tissue of the cotyledons and reduced endosperm; *e*, longitudinal section showing cotyledons extending about half the length of the ovule. *br*, Bract; *cv*, central vacuole of endosperm; *em*, embryo; *h*, hull; *mi*, middle septum; *o*, ovule; *pk*, packing tissue of ovary; *pl*, placenta; *sh*, shell; *st*, stigma; *tl*, cotyledon lobe.

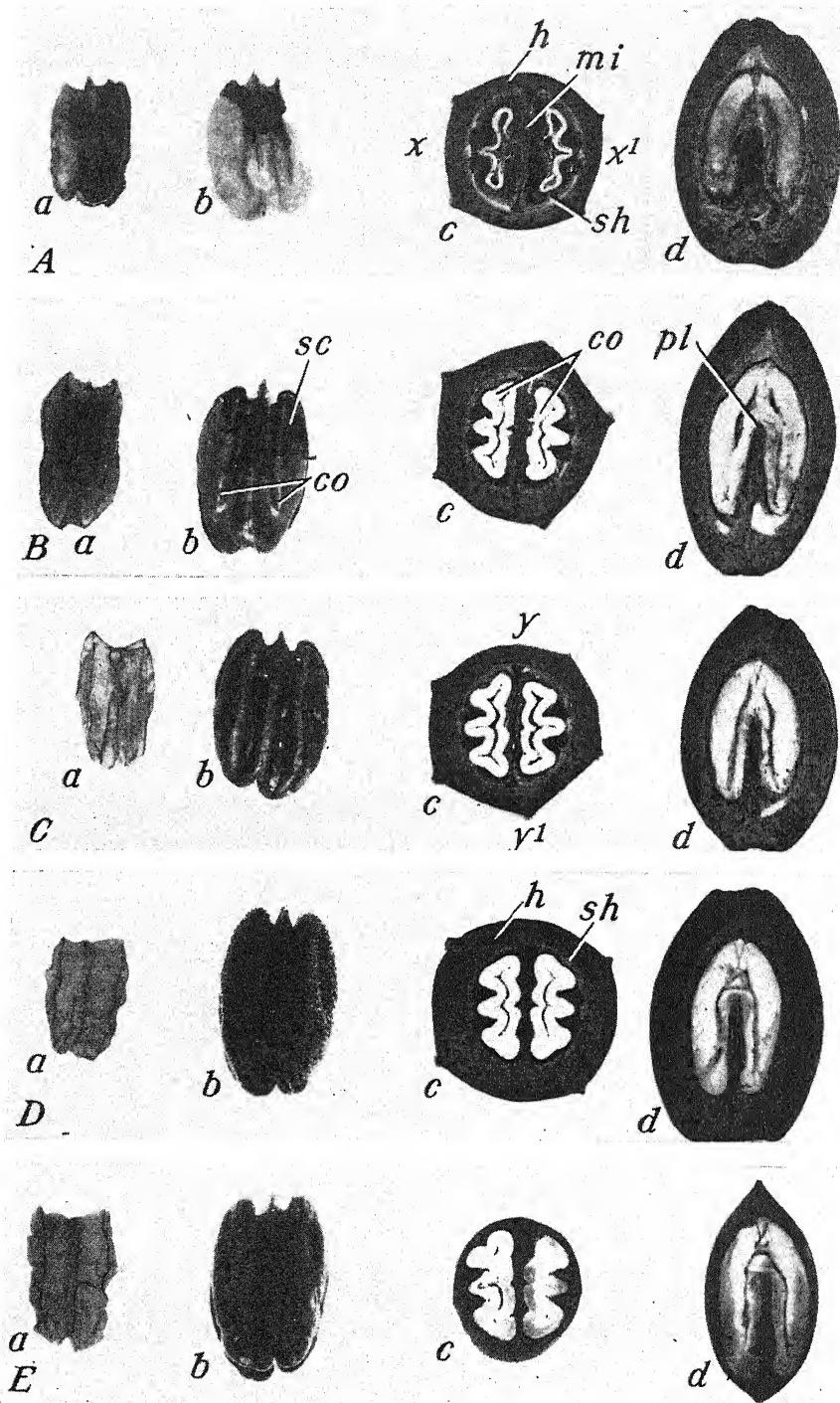


FIGURE 3.—For explanatory legend see opposite page.

Woodroof and Woodroof (13, p. 1057) stated that "Included within the folds of each cotyledon is a thin white membrane containing numerous vascular bundles." In the present work no vascular bundles were observed in the endosperm at any stage of its development. The tissues become crushed within the folds of the cotyledons in the later stages of filling, and the folds and creases thus developed superficially resemble vascular bundles (fig. 3, *D*, *a*, and *E*, *a*). However, close inspection of stained histological sections revealed no trace of vascular tissue and showed the endosperm to be a homogeneous and undifferentiated tissue (fig. 4, *A* and *B*).

#### EMBRYO GROWTH, OR FILLING EARLY STAGES

The first division of the zygote occurs about 42 days after pollination, or near the middle of July in Maryland. Two- to eight-celled embryos were observed in ovules fixed July 19 (fig. 1, *H*). Woodroof (15), working in Georgia, found that the first division of the zygote occurs about 56 days after pollination, and in an earlier work Woodroof and Woodroof (13) observed two- to four-celled embryos 70 days after pollination. Shuhart (10) did not record the exact time interval between pollination and zygote division but stated that the embryo begins to develop about September 1 at Stillwater, Okla. The details of tissue differentiation in very young embryos were not studied in the present work, emphasis being placed on the mechanism of the filling of the kernel in the later stages of embryo growth.

The embryo was a round mass of undifferentiated tissue in ovules collected on August 2, approximately 21 days after the first division of the zygote (fig. 1, *I*). A suspensor is formed in very young embryos but consists of only a few small cells that are soon crushed by the growing embryo (fig. 5, *C*). During late July and early August the basal portion of the ovule rapidly pushes downward into the ovarian cavity on either side of the middle septum. The fruit reaches approximately half its growth in size by the first of August (fig. 2, *A*, *i*).

The differentiation of cotyledons is evident in the embryos of fruits collected August 18; the meristems of the epicotyl and root axis are also recognizable (fig. 5, *C*). The embryo is still microscopic, however, and the much enlarged ovule is filled with the watery endosperm. By August 25, the embryo is large enough to be seen with the unaided eye (fig. 2, *B*, *a*, *d*). Growth in size of the ovule and fruit is almost complete at this time, and the tip of the shell at the blossom end of the fruit has begun to harden. For convenient reference the length of the

**FIGURE 3.**—Pecan-fruit parts showing endosperm absorption and embryo growth after the middle of September; X approximately 1. *A*, Dissected fruits collected September 15: *a*, Endosperm removed from within folds of the cotyledons and slightly stained in 1-percent safranine; *b*, kernel with seed coat mostly removed; *c*, cross section showing cotyledons somewhat thicker and endosperm more reduced than in figure 2, *D*; *d*, longitudinal section showing cotyledons extending almost to the basal end of the ovule. *B-D*, Dissected fruit collected approximately 1, 2, and 3 weeks later than those in *A*, showing further reduction of endosperm: *a-d*, Same as in *A*. *E*, Dissected mature nuts harvested November 7: *a*, Thin, membranous endosperm; *b*, kernel; *c*, cross section; *d*, longitudinal section. The stages between *D* and *E* were omitted because little change occurred in the kernels during this period. *co*, Cotyledon halves; *h*, hull; *mi*, middle septum; *pl*, placenta; *sc*, seed coat; *sh*, shell; *x-x'*, plane of the cotyledons (perpendicular to the middle septum); *y-y'*, plane of the middle septum.

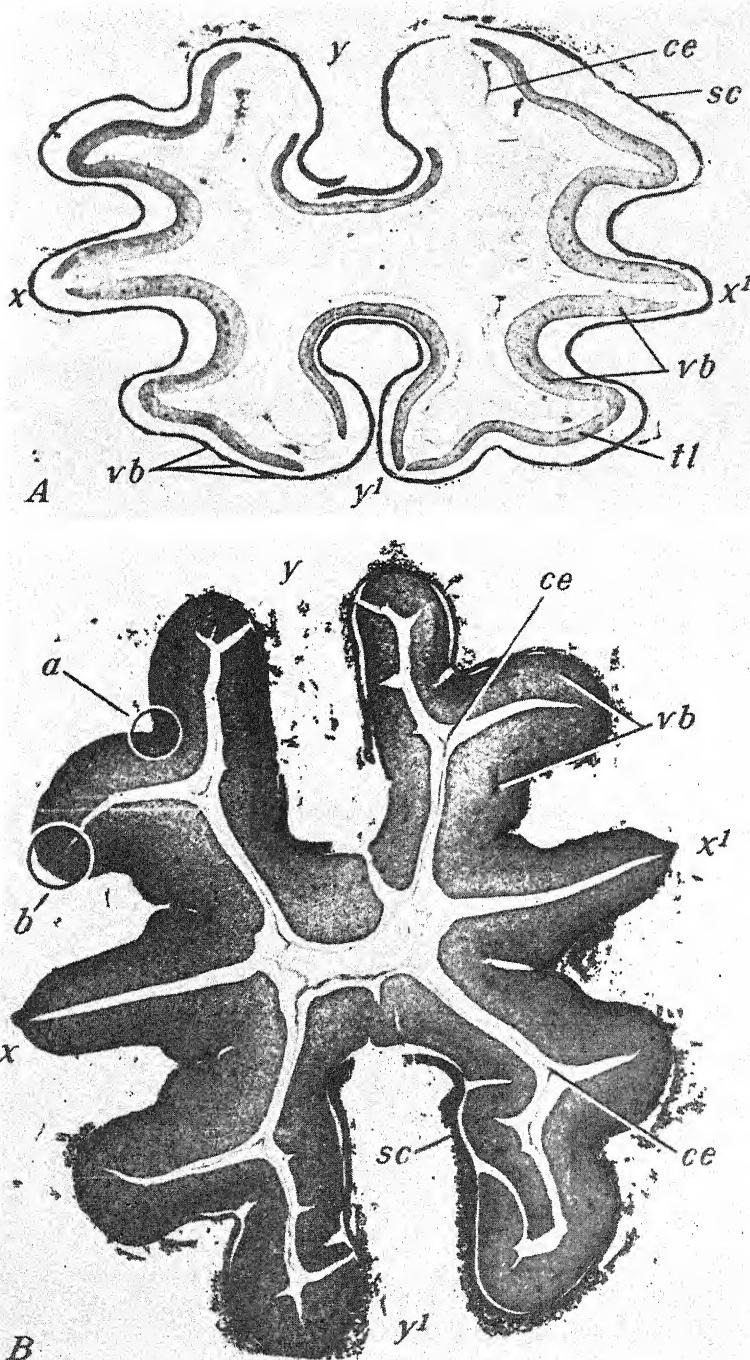


FIGURE 4.—For explanatory legend see opposite page.

embryo and other fruit and ovule structures at different dates during the season is given in table 1.

TABLE 1.—Length of fruit and seed structures of the pecan, variety Greenriver, at certain dates during the growing season of 1941, Beltsville, Md.

[Length given in millimeters]

Date	Period after pollination	Fruit	Ovule	Endosperm	Embryo
	<i>Days</i>				
June 1	0	8	0.5		
June 14	13	12	1	2-4 nuclei	Zygote.
July 12	41	20	4	2	Do.
July 19	48	25	5	3 1	2- to 8-celled. Many-celled.
July 26	55	30	6	5	0.5.
Aug. 9	69	40	15	14	
Aug. 25	85	40	25	25 (water stage)	2.
Sept. 8	99	40	30	Succulent-pad stage	15.
Sept. 22	113	40	30	Much reduced	30.
Nov. 7	<sup>2</sup> 159	40	30	Thin membrane	30.

<sup>1</sup> Cellular endosperm appeared.

<sup>2</sup> Mature nut.

#### GROWTH IN LENGTH OF COTYLEDONS

In Maryland the month of September is the period during which the most rapid embryo growth occurs. Fruits collected September 2 contained embryos 4 to 6 mm. long with bilobed cotyledons oriented perpendicular to the plane of the middle septum (fig. 2, C, a). The cotyledons elongate at first by means of a terminal meristem at the lower margin of the lobes (fig. 6, B). Meristematic activity is soon discontinued at the lower midpoint of each cotyledon, which forms an apical notch similar to that in the embryo of *Juglans regia* described by Nast (8). The apical meristem of the cotyledons is present in only very young embryos, and when the cotyledons are 2 to 4 mm. long meristematic activity is present throughout the tissue. From this point onward intercalary growth is responsible for rapid elongation of the cotyledons.

During the first half of September, growth of the cotyledons is largely in length rather than in thickness. The cotyledon halves follow the contour of the ovule in their downward growth and lie adjacent to the seed coat, thus enclosing and surrounding the endosperm. Each cotyledon is bisected by the middle septum, and the cotyledon halves grow into each lobe of the bilobed ovule. The four upper chambers of the ovule are filled by lateral folds of the cotyledon halves, which grow upward into these chambers as the main lobes of the cotyledons grow downward in the ovule.

FIGURE 4.—Median cross sections of pecan ovules (kernels);  $\times 7$ . A, 99 days after pollination, September 8. At this stage the cotyledons were elongating rapidly but had not yet reached the basal end of the ovule (see fig. 2, D, e). The cotyledon lobes were adjacent to the seed coat and enclosed the endosperm. B, 113 days after pollination, September 22. Thickening of the cotyledons was well advanced at this stage, and the endosperm was reduced to a small area within the cotyledon lobes. New tissue was being formed largely in subepidermal meristems (deeply stained) along the outer margins of the cotyledons adjacent to the seed coat. (See in fig. 6, A and C, respectively, enlargements of the encircled areas marked a and b.) ce, Cellular endosperm; sc, seed coat; tl, cotyledon lobe; vb, vascular bundles;  $x-x'$ , plane of the cotyledons;  $y-y'$ , plane of the middle septum.

Finch and Van Horn (5, p. 440) ascribed the early stages of filling to "the conversion of the gel into a layer of white solid material." Later a second "gel layer" is formed and this "in turn is converted into solid material which adds to and thickens the original solid layer"

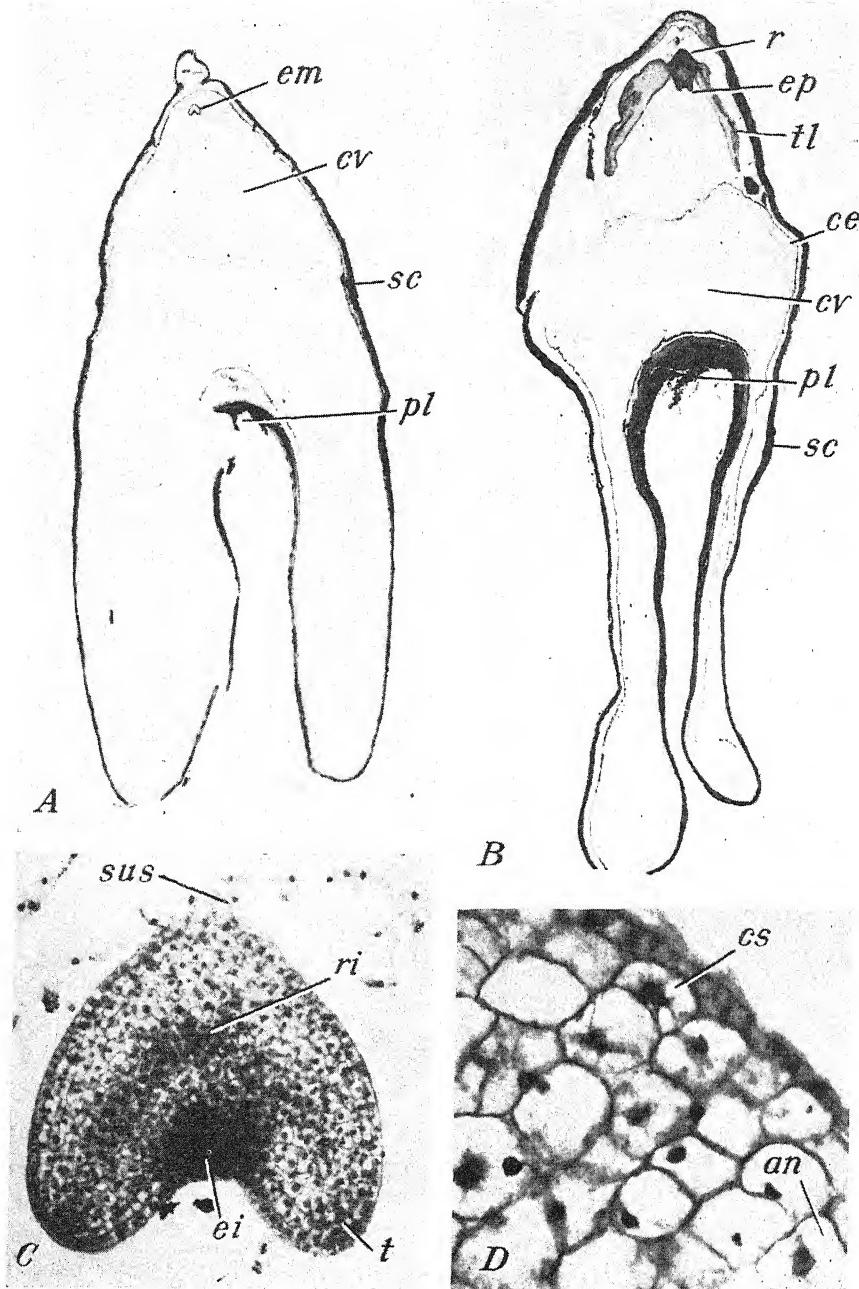


FIGURE 5.—For explanatory legend see opposite page.

(5, p. 442). It is easy to understand this account of the method of filling because of the rapidity with which the cotyledons extend into the lower part of the ovule. It will be noted in figure 2, *C* and *D*, and figure 3, *A*, that the cotyledons expand almost the full length of the ovule in 14 days. Furthermore, the cotyledons occupy a position adjacent to the seed coat and push the endosperm inward, thus giving rise to Finch and Van Horn's centripetal-layer explanation. It should be emphasized, however, that enlargement of the cotyledons is strictly an autonomous process and that increase in storage tissue is due to cell multiplication within rather than to a conversion process as proposed by Finch and Van Horn.

The cotyledons are relatively thin during the 14-day period of rapid elongation (fig. 2, *D*, *d*; fig. 3, *A*, *c*; and fig. 4, *A*). Cross walls of dividing cells are mostly perpendicular to the long axis of the cotyledon, resulting in elongation of the structure rather than thickening (fig. 6, *B*). The cells are highly vacuolate and have small nuclei typical of storage parenchyma (fig. 6, *D*). Nevertheless, cell division is fairly rapid and the mitotic figure is similar to that described for vacuolate cells by Sinnott and Bloch (11), except that cross-wall formation is accomplished by the cell-plate method. Cytoplasmic strands are prominent in these cells during the prophase of nuclear division (fig. 5, *D*).

Growth in length of the cotyledons apparently may be arrested by unfavorable nutritional conditions during the period of elongation. Nuts of the Mahan and Schley varieties with kernels showing varying degrees of shortening are illustrated in figure 7, *A*, *b*, *d*, and *D*, *b*. Some of these kernels are well-filled and solid even though they are shortened. Others, however, show air spaces within the folds of the cotyledon halves, indicating that cotyledon thickening did not progress to completion. In figure 7, *B*, *a*, is shown a cross section of a well-filled Mahan nut with full length kernel; in *B*, *b*, a shortened but solid, somewhat imperfect kernel; and in *B*, *c*, *d*, shortened poorly filled kernels. Although experimental evidence is not at hand to prove that it is true, it seems reasonable to believe that conditions of tree growth and nutrition at the time of cotyledon elongation may influence length of kernel.

FIGURE 5.—Sections of pecan ovules (kernels) and embryo tissue. *A*, Longitudinal section of ovule fixed 78 days after pollination, August 18. The embryo is microscopic and the endosperm is composed mostly of the watery central vacuole (compare with fig. 2, *A*, *k*). *B*, Longitudinal section of ovule fixed 15 days later than *A*, September 2. Lower portion of the ovule has collapsed because of rupture of the seed coat during dissection, allowing liquid of the central vacuole to escape. Peripheral cellular endosperm is more highly developed than in *A*, but still very fragile (compare with fig. 2, *C*, *d*). *C*, Embryo shown in *A*. The cotyledon lobes and the initials of radicle and epicotyl are in early stages of differentiation. *D*, Section of outer portion of cotyledon showing beginning of subepidermal meristem, September 3. The large, thin-walled cells have small nuclei and prominent cytoplasmic strands and have divided in a plane perpendicular to the surface of the cotyledon. *A* and *B*,  $\times 6$ ; *C*,  $\times 144$ ; *D*,  $\times 321$ . *an*, Cell in anaphase of division; *ce*, cellular endosperm; *cs*, cell with prominent cytoplasmic strand; *cv*, central vacuole of endosperm; *ei*, epicotyl initial; *em*, embryo; *ep*, epicotyl; *pl*, placenta; *r*, radicle; *ri*, radicle initial; *sc*, seed coat; *sus*, remains of suspensor; *t*, cotyledon initial; *tl*, cotyledon lobe.

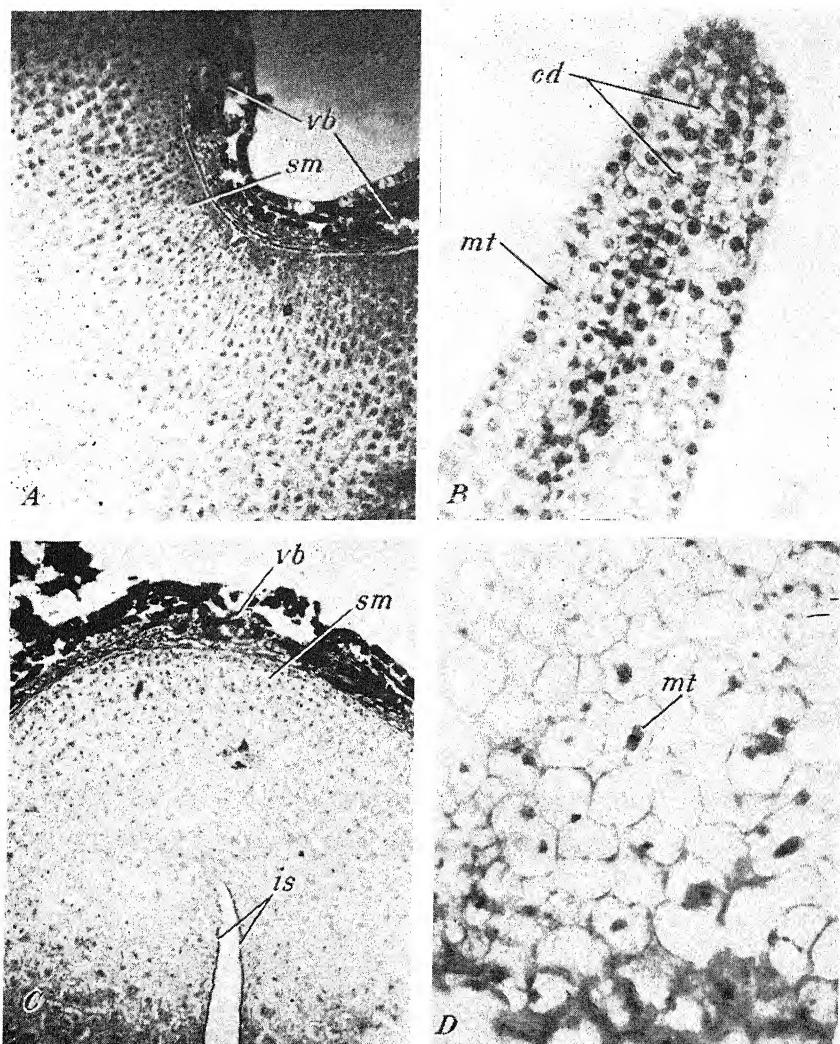


FIGURE 6.—Sections of pecan cotyledons showing meristems and cell structure. *A*, Cross section of a portion of outer surface of cotyledon opposite an inward fold of the seed coat, showing well-developed subepidermal meristem, September 22 (position of the sector is indicated by *a* in fig. 4, *B*). Tissue of the cotyledon was in contact with the seed coat from which nutrients were derived by absorption. *B*, Longitudinal section through lower edge of cotyledon in 2-mm. embryo, August 23. Rapidly dividing cells with large nuclei characteristic of meristematic tissue occurred on the lower margins (upper in photograph) of cotyledons 1 mm. long; most of the cells had divided in a plane perpendicular to the long axis of the cotyledon, resulting in elongation. *C*, Cross section through portion of cotyledon showing greater meristematic activity on the outer surface than on the inner, September 22 (position of the sector is indicated by *b* in fig. 4, *B*). *D*, Cross section showing cell division in the inner tissue of the cotyledon during the period of elongation, September 2. The cells were highly vacuolate and typical of storage parenchyma, but division was continuous throughout the tissue. *A* and *C*,  $\times 57$ ; *B* and *D*,  $\times 259$ . *od*, Daughter cells with cross walls perpendicular to the long axis of the cotyledon; *is*, inner surface of cotyledon; *mt*, cell in metaphase of division; *sm*, subepidermal meristem of cotyledon; *vb*, vascular bundles of seed coat.

## GROWTH IN THICKNESS OF COTYLEDONS

Growth in thickness of the cotyledons is accomplished by the activity of a subepidermal meristem (fig. 5, *D*; and fig. 6, *A, C*), beginning at the time the cotyledons reach full length the first or second week in September. Nast (8, p. 189), in discussing the thickening of cotyledons in *Juglans regia*, stated:

In fifteen-millimeter embryos a rather pronounced growth in thickness begins in the area nearest to the epicotyl and spreads to the margins. This increase in thickness is carried on by a cambium-like meristem.

She found (*pp. 189-190*) that "Storage of food begins with increase in thickness of the cotyledons and starts in the basal region."

Growth in thickness of full-length pecan cotyledons is similar to that of embryos of *Juglans regia* described by Nast (8). In the early stages of thickening, activity of the subepidermal meristem is greater on the inner surface of the cotyledons adjacent to the endosperm, presumably as a result of the nutritive action of the endosperm. During the later stages of filling when the endosperm is mostly absorbed, cell division is more active in the outer meristem of the cotyledons (fig. 6, *C*). At this time food materials move into the embryo along the outer margins by absorption from the seed coat. Location of meristem activity in the cotyledons thus seems to be related to location of the nutrient supply. The formation of solid kernels depends upon the complete filling of the cavity within the folds of the cotyledon halves, and in well-filled kernels meristematic activity continues in the outer and to some extent in the inner meristem until almost harvesttime.

Storage of food is greatly accelerated as the cotyledons increase in thickness. Cells in the central region of the cotyledons first show the presence of storage products, and as thickening progresses cells nearer the margin become filled with stored food. In the final stages of cotyledon thickening abundant stored food is found in the dividing cells of the meristem near the surface.

Elaborated food is translocated to the embryo by absorption from the seed coat. The embryo is probably nourished almost entirely by the endosperm until thickening of the cotyledons is under way. The seed coat has a well-developed vascular system composed of a network of bundles, which extend from the placenta to all parts of the integument (fig. 4, *A*; fig. 6, *A, C*). The amount of elaborated food available from the tree that moves through the vascular system of the seed coat to the embryo seems to be the factor which determines the filling of the kernel rather than the conversion of gel to solid tissue as suggested by Finch and Van Horn (5). Poorly filled nuts are therefore an indication that the tree is unable to supply the materials necessary for complete embryo development.

No biochemical tests were made in this work to determine the nature of storage products in the pecan seed. Finch and Van Horn (5) found that the gel layer, or endosperm, contained invert sugars but no fats, whereas the embryo contained abundant fats but no sugars. Thor and Smith (12) reported that a sharp maximum in the concentration of total sugars in the nut occurred in July and suggested that this may be related to zygote division, which, according to Woodroof and Woodroof (13), begins about this time. The high sugar content of the nut in Texas during July is undoubtedly related to rapid endosperm

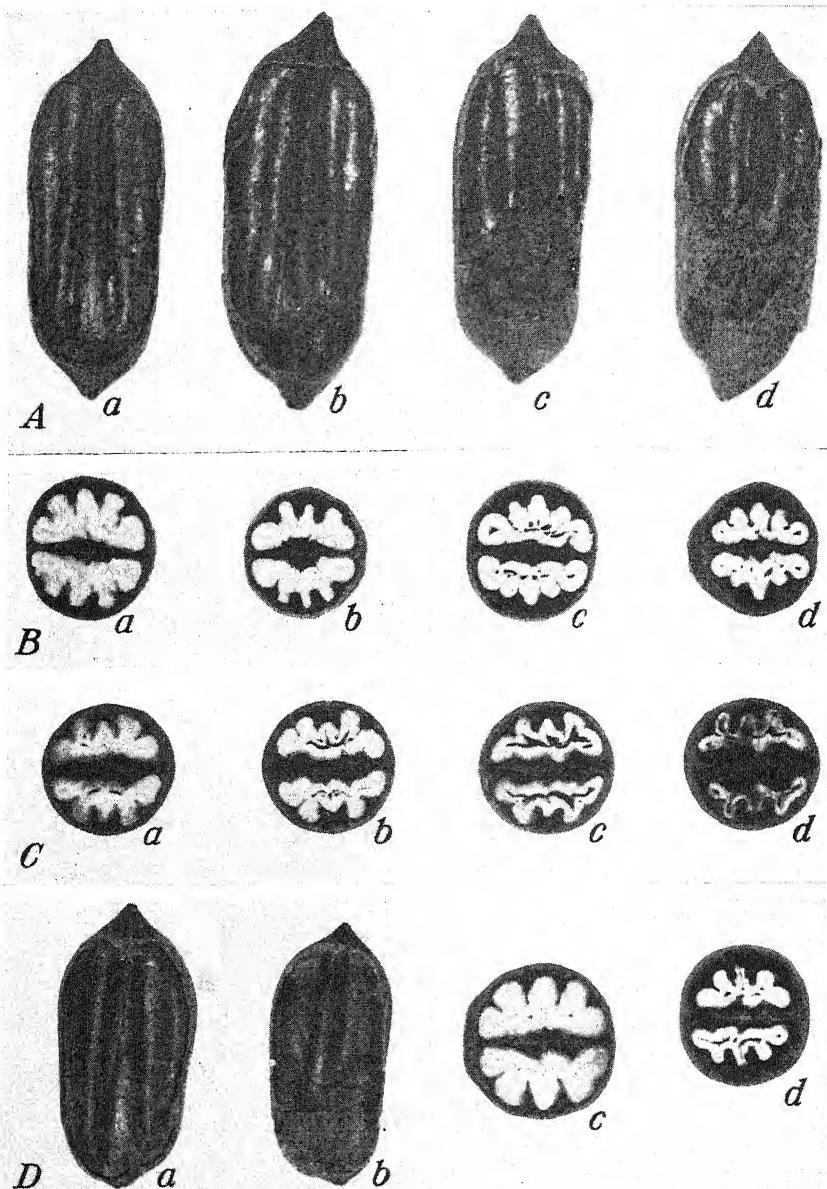


FIGURE 7.—Mature pecan nuts showing different types of filling;  $\times$  approximately 1. *A*, Mahan nuts; *a*, Well-filled nut; *b-d*, nuts with shortened kernels. *B*, Cross sections of nuts shown in *A*; note in *a* and *b* that the kernels are solid and well-filled, whereas in *c* and *d* they are hollow and poorly filled. *C*, Stuart nuts: *a*, Well-filled nut; *b-d*, poorly filled nuts showing spaces resulting from lack of cotyledon thickening. *D*, Schley nuts: *a*, Well-filled nut; *b*, nut with shortened kernel; *c* and *d*, cross sections of well-filled and poorly filled nuts.

development rather than to the beginning of embryo growth as suggested by Thor and Smith (12). From these reports it seems clear that the endosperm is composed largely of carbohydrate material. The fact that fats appear in the embryo only after development is well under way indicates that fat synthesis takes place in the cotyledons. This is probably true of proteins as well. While it is not the purpose of this paper to discuss the physiology of nut filling, the normal course of ovule development indicates that elaborated food in soluble form is translocated to the endosperm and embryo through the elaborate vascular system of the seed coat. In the later stages of filling, the cotyledons absorb soluble food materials from the seed coat and these are transformed within the tissue of the cotyledons into fats and protein, the principal storage products of the kernel. This concept of the movement of translocated foods agrees with the statement of Thor and Smith (12, p. 118) that ". . . practically all of the oil content of the pecan kernel is formed from materials brought into the fruit from other parts of the tree at the time of oil formation." Woodroof, Woodroof, and Bailey (14, p. 38) also concluded: "Unfilled or poor quality nuts are directly related to unfavorable growing conditions the last month before harvest."

The morphological details of tissue differentiation in the hypocotyl and epicotyl were not determined in this study, because these structures are small and have no significance as storage organs.

#### DISCUSSION

One of the most striking features of pecan-nut development is the long period of endosperm enlargement before there is visible evidence of embryo growth. The first division of the zygote occurs about 28 days after the endosperm starts to grow and about 42 days after pollination. During this period of zygote dormancy the failure of initiation of endosperm development or of its continuation would undoubtedly result in dropping of the fruit. The large increase in drop of nuts under cellophane bags as compared with that of unbagged nuts observed by Crane et al. (4) during the early periods of nut development may possibly be explained as the result of endosperm failure. In this case the unfavorable environment within the bags (high temperature and humidity) probably caused the delicate endosperm to cease development and the fruits to drop. The unbagged clusters showed the greatest rate of drop during August and September at the height of endosperm development and after embryo growth was well under way. During this period the endosperm is composed of a layer of fragile cellular tissue surrounding a watery central vacuole. The thin-walled cells of the cellular endosperm are undoubtedly easily ruptured, and unfavorable environmental influences, such as hard winds and high temperature, could cause injury to a few of these cells. Oxidation of this tissue, indicated by discoloration, undoubtedly spreads rapidly after such injury and causes the nut to

drop. Crane et al. (4) observed that dropped nuts showed internal discolored areas which appeared to be ruptured tissues, and they concluded as follows (p. 42):

The evidence indicates that the nuts are most susceptible to injury from external influences during the period from about June 1 to September 1, when they are in the watery stage, and that these external factors are most injurious when they alter the environment most rapidly.

While endosperm failure is difficult if not impossible to demonstrate experimentally it seems clear that this may be one of the large factors in nut drop, at least in the first half of the growing season.

Brink and Cooper (1, 2) described a type of seed failure in alfalfa caused by the inability of the endosperm to keep pace with growth in the surrounding tissues of the seed. As a result, the maternal tissue at the chalazal end of the embryo sac produces abnormal growth that causes collapse of the ovule. This type of failure, which is termed "somatoplasic sterility," occurred in 34.4 percent of self-pollinated ovules as compared with only 7.1 percent of cross-pollinated ones. The greater percentage of seed set after cross-pollination is attributed to the added impetus to growth of the endosperm resulting from cross-fertilization, or hybridity. Brink and Cooper (1, 2) stated that this type of seed failure is probably a widespread, although rather obscure, form of sterility in plants. Since pecan, like alfalfa, is adapted to cross-pollination, the type of endosperm failure which they described may cause some of the drop of pecan fruits early in the season. Self-pollination probably occurs rather frequently in certain varieties, especially in certain seasons when dichogamy is not very pronounced, but little information is available on this point. Unpublished results indicate that the set of nuts in certain varieties after self-pollination is much smaller than after cross-pollination, and many of the self-pollinated nuts are empty at maturity. Further study of nuts that drop after artificial self-pollination and those that drop from natural causes should be made to determine whether or not endosperm failure is responsible for the rather heavy drop of nuts that frequently occurs during the first half of the growing season.

One of the most probable causes of endosperm failure is the high degree of turgidity that persists within the ovule throughout endosperm development. The tension set up by the large watery vacuole undoubtedly causes the peripheral cellular tissue to be more susceptible to injury from environmental influences. Crane et al. (4, p. 39) reported that "Schley nuts sometimes split open when the developing kernel is in the 'watery stage' . . . after a period of drought followed by heavy rains." In this case the pressure developed in the endosperm as a result of water becoming suddenly available was so great as to cause the longitudinal rupture of the seed coat, shell, and shuck and the release of the liquid endosperm. Such injured nuts drop within 3 to 5 days. On the other hand, a period of continued drought at any time during the period of development is likely to affect the size or filling of the nuts or both because moisture is withdrawn and withheld from the nuts by the leaves during periods of excessive transpirations. It is of the greatest importance, therefore, that a uniform moisture supply be available to the tree during the main period of nut growth and filling in order that the nut may attain good size and the tissues of the endosperm and embryo may develop at an optimum rate.

It should be borne in mind that the principal function of the endosperm is to provide nutrients for the initial stages of embryo growth. The nutrients absorbed from the endosperm by the embryo are used largely for the production of new tissue rather than for storage, especially during the early period of embryo growth when the cotyledons are elongating. Nutrients used in the storage of food are derived principally from the tree by absorption via the seed coat. Thus, under normal conditions the endosperm provides the necessary nutritional environment for the first stages of embryo growth, but because of its fragile, turgid qualities it also may be the cause of nut failure.

The most important changes in embryo development that affect the formation of well-filled kernels are the last stages of cotyledon thickening, which occur 14 to 21 days prior to harvest. Finch and Van Horn (5, p. 442) concluded:

The rate at which the filling process proceeds, i. e., the rate at which the carbohydrate containing gel is formed in the nut and converted to fats, bears an important relation to the character of filling attained at harvest time.

They outlined the growth-rate conditions under which well-filled kernels and poorly filled kernels may be produced. The writer believes that Finch and Van Horn overestimated the importance of the endosperm in its relation to cotyledon thickening. It is probable that the carbohydrate-containing endosperm nourishes the embryo in its early stages of growth as it does in most seeds. However, the endosperm in the pecan ovule is largely absorbed before cotyledon thickening is complete, as is shown in figure 3, C, a and c. It seems highly improbable that the enormous quantities of stored food translocated to the cotyledons during the later stages of filling are derived from the endosperm through the process of conversion of gel to solid material, as suggested by Finch and Van Horn. The endosperm is so greatly reduced during the last month of filling as to be of little nutritional significance. This final period of cotyledon thickening is the most important phase of nut filling, as the production of solid kernels at maturity depends upon the complete filling of the cavity within the folds of the cotyledon halves.

The important point here is that sufficient elaborated food must be available from the tree directly, or indirectly from temporary reserves in other parts of the fruit, to fill all of the storage cells developed by the subepidermal meristems. The meristems of the cotyledons function in direct response to the nutrient supply, because the inner meristem is more active at first while the endosperm is still of nutritional significance and the outer meristem builds most of the tissue of the cotyledons during the later stages of filling when nutrients are absorbed from the seed coat. In view of this relation between nutrients and meristem activity, it seems likely that short kernels may be due to poor nutritional conditions in the tree at or prior to the time of cotyledon elongation. Thus, both elongation and thickening of the kernel may be affected by the food supply and the water needed to provide favorable conditions for translocation from the tree.

Finch and Van Horn (5, p. 445) stated:

The problem of reducing or eliminating nuts having the hollow kernel at harvest time is then one of providing for a rapid and continuous progression through the stages of filling. The relation of carbohydrate supply to filling seems of

increased importance now with direct evidence that the solid content of the kernel is formed by conversion from a carbohydrate material that is successively formed within the kernel.

Finch and Van Horn were correct in concluding that filling should be rapid and continuous, but they were in error as to the method of kernel formation. The cotyledons are not formed by conversion from a carbohydrate material (endosperm), but they develop in both length and thickness by means of internal cell division and cell enlargement. During the later stages of filling, stored food enters the tissue of the cotyledon by absorption from the seed coat, since the endosperm at this time is so greatly reduced as to be of little nutritional significance. Finch and Van Horn were correct in stating that the problem of eliminating nuts having the hollow kernel at harvest is one of providing a continuous and adequate carbohydrate supply, but in the later stages of filling this supply moves directly to the cotyledons from the seed coat instead of first appearing as endosperm and later being converted to cotyledonary tissue.

#### SUMMARY

Pistillate flowers of the pecan are pollinated about June 1 in Maryland, and union of the egg and male gamete occurs about 4 days later. Union of the polar-fusion nucleus and the second male gamete from the pollen tube to form the primary endosperm nucleus likewise takes place a few days (4) after pollination.

The development of the fruit may be divided into two periods characterized as follows: (1) Period of endosperm development, which continues during the second half of June and July and August; and (2) period of embryo growth, which occurs during September, October, and the first week of November. The beginning of shell hardening at the tip of the nut is a morphological feature that separates approximately the two periods.

Endosperm growth begins 14 days after pollination and the endosperm is free-nucleate until about August 1. A large central vacuole filled with liquid is characteristic of the endosperm throughout its development. Beginning early in August, a layer of cellular endosperm, jellylike in nature, is laid down on the outer margins of the central vacuole and adjacent to the seed coat. In September the endosperm is rapidly surrounded and absorbed by the folds of the cotyledons.

Embryo growth begins 42 days after pollination with the first division of the zygote about July 12. By the last of August, the embryo is about 2 mm. long and large enough to be seen with the unaided eye. Growth in size of the ovule and fruit is about complete at this time, and the tip of the shell at the blossom end of the fruit has begun to harden.

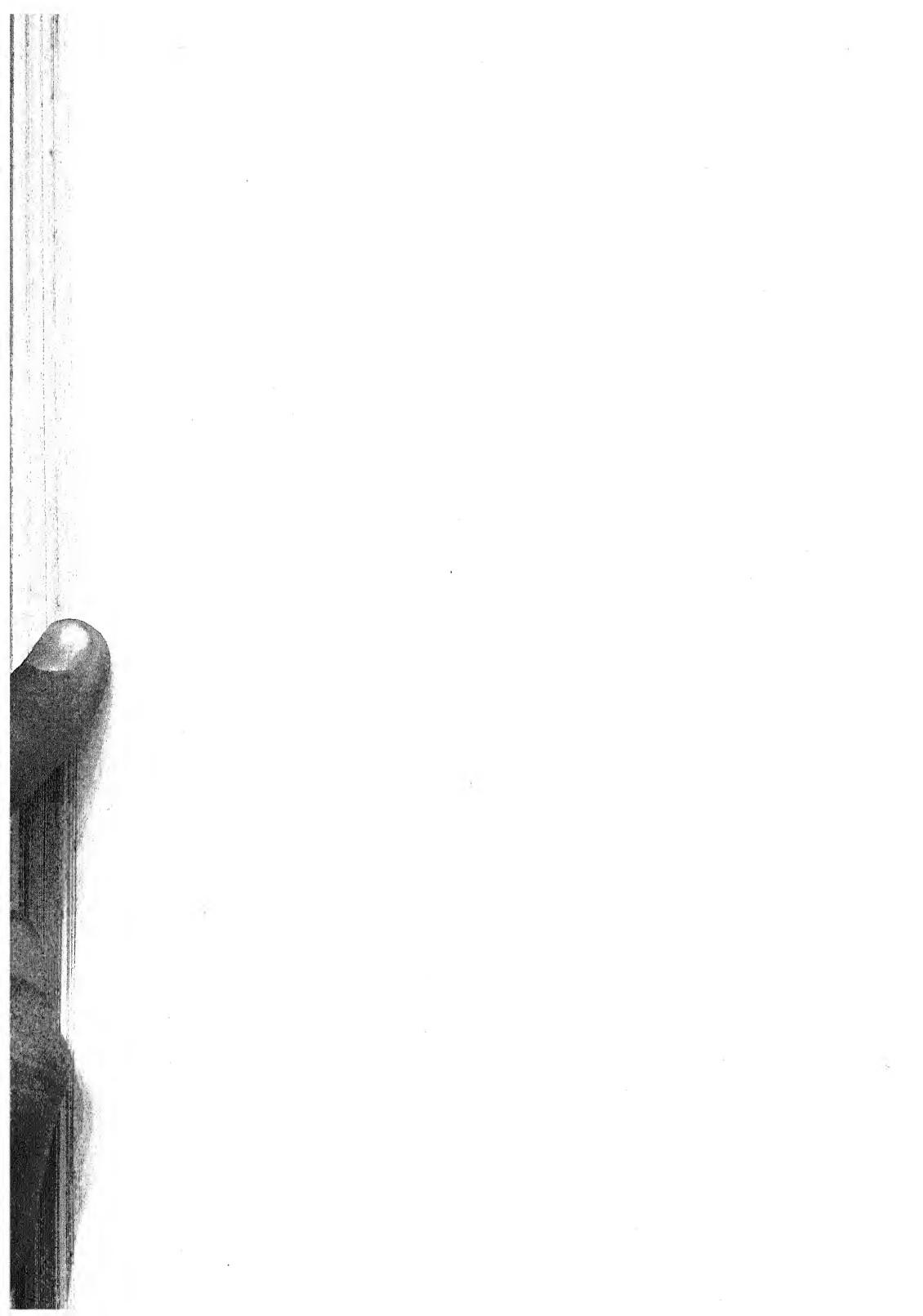
The cotyledons elongate into the lower portion of the ovule during the first half of September.

The most important phase of pecan-nut filling is thickening of the cotyledons, which occurred in Maryland between the middle of September and harvest. New tissue is formed by a subepidermal meristem, at first on the inner surface of the cotyledons while the endosperm is supplying nutrients and later on the outer surface during which time nutrients are supplied through the adjacent seed coat. In kernels that

are well-filled and solid, the subepidermal meristem remains active and builds new cells for storage tissue until almost harvesttime. Since meristem activity in the cotyledons seems to function in response to nutrient supply, the formation of plump, solid kernels depends upon the translocation of adequate quantities of food materials from the tree through the seed coat to the embryo during the last month of filling.

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## APPARENT DIGESTIBILITY BY SHEEP OF LIGNIN IN PEA AND LIMA-BEAN VINES<sup>1</sup>

By R. E. DAVIS, *nutritionist*, C. O. MILLER, *junior chemist*, and I. L. LINDAHL,  
*junior chemist*, Bureau of Animal Industry, Agricultural Research Adminis-  
tration, United States Department of Agriculture<sup>2</sup>

### INTRODUCTION

This paper reports the results of the second part of a study of the composition and apparent digestibility by sheep of pea and lima-bean vines<sup>3</sup> that were preserved by artificial dehydration. The first part of the investigation was concerned primarily with hemicellulose and related compounds (11);<sup>4</sup> this paper has to do with the apparent digestibility of lignin.

### WORK OF OTHER INVESTIGATORS

It is generally accepted that lignin is the least digestible portion of the structural constituents of plant material (7). Some investigators claim that lignin is in part digested by animals, others maintain that it is undigested. Csonka and coworkers (4) concluded, from an experiment in which alkali lignin was fed to cows and dogs, that lignin was at least partly broken down by the digestive processes of the animal body. Maynard (9) found that digestion of lignin by rabbits and guinea pigs fed alfalfa hay was practically nil, but that a lamb digested 28 percent of the lignin from the same hay. Louw (7) reported that with sheep the digestibility coefficient of lignin was 24.5 in grass of 1 month's growth, whereas in grass of 4 months' growth it was only 11.6. In a later experiment in which hay was fed to sheep at daily levels of 600, 800, and 1,000 gm., Louw (8) found that the digestibility coefficients were 15.6, 12.4, and 16.4, respectively. Lancaster (6) obtained digestibility coefficients ranging from -40.5 for a sample of rape to +32.4 for turnips, in a series of metabolism trials with sheep. In a metabolism experiment in which several different forage plants were fed to sheep, Bondi and Meyer (1) obtained di-

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<sup>3</sup> As used in this paper the terms "pea vines" and "lima-bean vines" include the vines and empty pods obtained as byproducts from canneries.

<sup>4</sup> Italic numbers in parentheses refer to Literature Cited, p. 287.

gestibility coefficients that ranged from 35.1 to 64, indicating that the sheep digested lignin comparatively well. Among the investigators who reported that lignin is indigestible are Rogozinski and Starzewska (12) and Naumann (10). Crampton and Maynard (3) recovered 97.8 and 99.3 percent of the dietary lignin in the feces of rabbits and a steer, respectively, and in a later paper Crampton (2) reported that lignin was not only poorly digested but also that it interfered with the digestibility of other constituents of the plant material.

#### MATERIAL AND METHODS

The pea and lima-bean vines used in this study were obtained from a cannery in Pennsylvania. They were dehydrated in a commercial dryer and shipped to the Agricultural Research Center at Beltsville, Md.

Lignin determinations were made by the method of Davis and Miller (5). This method includes both enzymatic and chemical treatment. The material was first extracted with ether and then digested by pepsin, clarase, and trypsin. The residue from these digestions was analyzed for lignin by the 72-percent sulfuric acid method. The analytical procedure was the same for both feed and feces. Complete analyses for pea and lima-bean vines were reported in the previous paper (11).

The feeding trials were conducted during the winter of 1942. Four yearling and 2-year-old Hampshire ewes were used. During a preliminary period alfalfa hay was mixed with the pea and lima-bean vines, but the amount of alfalfa hay was gradually reduced until the entire feed consisted of pea or lima-bean vines. The animals were placed in metabolism cages a few days prior to the experimental period and confined there throughout the test. The experimental period was 10 days. Details of the procedure are given in the previous paper.

#### EXPERIMENTAL RESULTS

The lignin content of the lima-bean vines was slightly greater than that of the pea vines, the average lignin content being 6.42 and 6.05 percent, respectively. Owing to the fact that the sheep picked over the feed and left the coarser parts, the lignin content of the refused feed was higher in both cases than that of the original feed. This effect was more pronounced in the refused pea vines, which had an average lignin content of 8.2 percent as compared with 7.6 percent for the refused lima-bean vines.

The digestibility data are given in table 1. As shown in the table, the lignin in pea vines was digested more readily by sheep than the lignin in lima-bean vines. In every trial when the same sheep was fed both feeds, the digestibility of the pea-vine lignin was greater. Incidentally, as shown in the table, the average digestibility of the dry matter in the lima-bean vines was slightly higher than in the pea vines.

TABLE 1.—*Digestibility by sheep of dry matter and lignin in pea and lima-bean vines*<sup>1</sup>

Pea vines			Lima-bean vines		
Item	Dry matter	Lignin	Item	Dry matter	Lignin
Sheep No. 6V:			Sheep No. 6V:		
Fed.....grams.....	16,000	924.8	Fed.....grams.....	16,000	1,012.8
Consumed.....do.....	13,046	693.8	Consumed.....do.....	11,409	651.9
Digested.....do.....	8,274	105.9	Digested.....do.....	7,386	27.9
Digested.....percent.....	63.4	15.26	Digested.....percent.....	64.7	4.28
Sheep No. 17U:			Sheep No. 17U:		
Fed.....grams.....	16,000	924.8	Fed.....grams.....	16,000	1,012.8
Consumed.....do.....	11,218	554.2	Consumed.....do.....	9,886	567.1
Digested.....do.....	7,354	89.4	Digested.....do.....	6,341	64.4
Digested.....percent.....	65.5	16.13	Digested.....percent.....	64.1	11.36
Sheep No. 18V:			Sheep No. 18V:		
Fed.....grams.....	16,000	1,011.2	Fed.....grams.....	16,000	1,040.0
Consumed.....do.....	13,795	820.5	Consumed.....do.....	12,590	784.6
Digested.....do.....	8,477	132.4	Digested.....do.....	7,908	121.2
Digested.....percent.....	61.4	16.14	Digested.....percent.....	62.8	15.45
Sheep No. 42V:			Sheep No. 19U:		
Fed.....grams.....	16,000	1,011.2	Fed.....grams.....	16,000	1,040.0
Consumed.....do.....	12,045	671.9	Consumed.....do.....	8,817	484.0
Digested.....do.....	7,568	117.2	Digested.....do.....	5,976	55.6
Digested.....percent.....	62.8	17.44	Digested.....percent.....	67.8	11.49
Average percent digested by the 4 sheep.	63.3	16.2	Average percent digested by the 4 sheep.	64.8	10.6

<sup>1</sup> Included pods.

As shown also in the table, the sheep consumed a smaller quantity of lima-bean vines than of pea vines, presumably because the stems of the former were coarser and more woody. Less lignin was consumed in the lima-bean vines than in the pea vines in spite of the fact that the percentage of lignin in the former was slightly higher.

## SUMMARY

In a study conducted at the Agricultural Research Center, Beltsville, Md., pea vines and lima-bean vines (including the empty pods) obtained as byproducts from a cannery were fed in the dehydrated state to four yearling and 2-year-old Hampshire ewes. The apparent digestibility of lignin in each of these products was determined in a 10-day test period during which these feeds were given to the same group of animals. The digestion coefficients of the lignin in the pea vines was 16.2 percent and that in the lima-bean vines, 10.6 percent.

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## RHIZOCTONIA FOLIAGE BLIGHT OF SUGAR BEETS<sup>1</sup>

By JOHN E. KOTILA

Senior pathologist, Division of Sugar Plant Investigations, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture

### INTRODUCTION

In July 1938, after a rather prolonged period of rainy weather, a previously unrecognized disease affecting the foliage of sugar beets (*Beta vulgaris L.*) was found in experimental plantings of the Department of Agriculture at Arlington Experiment Farm, Arlington, Va. The disease occurred in the experimental plots there in the 1939, 1940, and 1941 seasons and since then each year in the experimental plantings of sugar beets at the Plant Industry Station, Beltsville, Md. It was rather difficult to find affected plants after an extended period of dry weather. Observations during the past 5 years have shown that the disease occurs also rather generally in Michigan, Illinois, Wisconsin, and Minnesota. In 1945 it was found in an irrigated field at Las Animas, Colo. High incidence and severe effects of the disease appear to be directly associated with prolonged periods of high humidity.

In a brief report (10) <sup>2</sup> foliage blight was attributed to a strain of *Rhizoctonia solani* Kühn which has a distinctly different pattern of attack from that associated with the *Rhizoctonia* strains that cause sugar-beet crown and root rot and dry rot canker. So far as the writer knows, this disease has not been described elsewhere. In 1924 Schenck (22), however, reported the occurrence of a *Hypochnus* that produced a cobweblike mycelium on the surface of sugar-beet leaves in Germany. Apparently this fungus was almost entirely superficial, since Schenck found no evidence of penetration of the leaf tissue and did not mention any necrotic spotting of leaves. It produced basidia chiefly on the lower side of the leaves. From a study of spore characters Schenck suggested (22, p. 322) that the fungus be called either *Hypochnus betae* or "*Corticium vagum* B. and C. var. *betae*," but she gave no formal description. Her inoculation experiments with potatoes (*Solanum tuberosum L.*) and sugar beets did not result in infection. This organism is of interest because of its fruiting habit, but its lack of pathogenicity makes it distinct from the strains of *Rhizoctonia solani* that cause sugar-beet foliage blight in the United States.

<sup>1</sup> Received for publication October 22, 1946.

<sup>2</sup> Italic numbers in parentheses refer to Literature Cited, p. 313.

After the discovery of sugar-beet foliage blight cultural and pathogenicity studies were made to determine the identity of the causal organism, its mode of dissemination, its range of pathogenicity, and the nature of the strains that exist within the organism. This paper reports the results of these studies.

#### DESCRIPTION AND PROBABLE COURSE OF FOLIAGE BLIGHT IN THE FIELD

Characteristic symptoms of foliage blight are found on sugar-beet plants that have reached the 6- to 10-leaf stage and on larger plants. The foliage-blighting *Rhizoctonia* is commonly manifested by attacks on the heart leaves. These it reduces almost to stubs consisting of the petiole with distorted portions of the leaf blade and blackened fragments of diseased tissue (fig. 1). It produces shallow, brown or

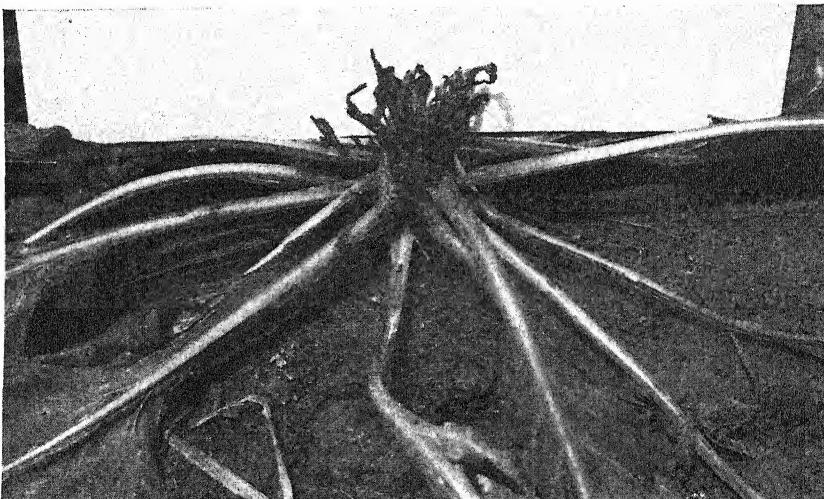


FIGURE 1.—*Rhizoctonia* foliage blight on the heart leaves of a sugar-beet plant, where it causes necrosis of petioles and blackened margins and tips of leaf blades. Note the shallow cankers on some of the petioles.

black cankers on the petioles, causing them to become distorted. Continued attacks may suppress or retard the growth of the heart leaves. In dense stands such blighted plants may shortly be overgrown by neighboring plants and be overlooked. Blighting effects are seldom observed on the taller leaves in the outer whorls of sugar-beet plants before July.

After a period of wet weather the disease appears on the larger leaves as irregular, blighted areas, sometimes involving a third to a half of the fully expanded leaf blade (fig. 2). For a brief period the affected areas have a water-soaked appearance. Then they become dark brown and finally black. One or more leaves of a plant may be affected. At relative humidities approaching 100 percent and with temperatures of 21° to 25° C. the disease progresses rapidly. Under less humid conditions and with lower temperatures the growth of the

causal fungus is confined to the crown region of the sugar-beet plants, where it causes necrosis of the tips and margins of the young leaves. As the weather becomes warmer, periods of high humidity set up conditions favorable for the development of the basidiospore stage of the organism. This stage, which consists of a powdery, grayish-white growth, is found almost exclusively on the ventral side of the

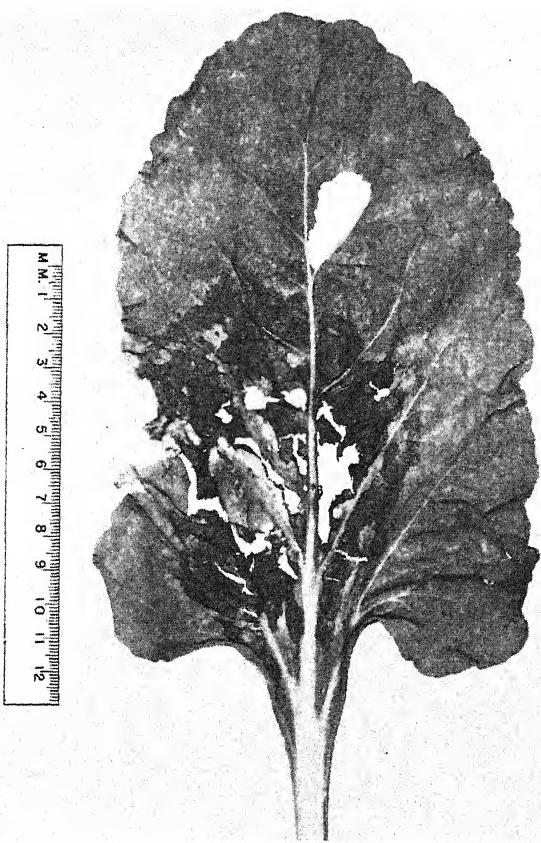


FIGURE 2.—Fully expanded leaf blade, showing large, necrotic area caused by foliage-blighting *Rhizoctonia*. Note that on drying the affected areas become brittle, fall out, and leave holes that resemble injury by hail or by certain insects.

leaves. It develops chiefly on sound tissue adjacent to necrotic areas and on sound portions of petioles adjacent to the black cankers (fig. 3). The perfect stage of the foliage blight fungus is very delicate and of rather short duration.

As indicated, the formation and release of the basidiospores are dependent upon warm, humid weather. By the basidiospores the fungus is disseminated for a considerable distance, possibly 100 feet or more. Under favorable conditions the basidiospores germinate within 12 hours and penetrate the sugar-beet leaf, where the growth

of the mycelium is very rapid. From the place of initial infection, usually a necrotic area about 5 mm. in diameter, the mycelium ramifies on the surface of the leaf and hyphal tips enter the leaf here and there through the stomata or penetrate directly after forming infection cushions. With the rapid invasion of the parenchyma several infection areas may coalesce, the area of involvement may increase, and the conspicuous leaf blight may result. The chlorophyll of the parenchyma cells is soon completely destroyed by the *Rhizoctonia*, and only the transparent cuticle of the leaf is left unaffected. The perfect stage of the fungus may again be formed, and the release of basidiospores may bring about secondary dissemination. Contact of a diseased leaf with a healthy one may result in direct infection of the latter, and some extension of the fungus from a diseased plant to its neighbors may also be brought about by the contact of leaf blades or petioles.



FIGURE 3.—Sugar-beet petiole, showing the basidial stage of the *Rhizoctonia* that causes foliage blight.  $\times 6$ .

In the field several slightly affected plants may be found sometimes in immediate proximity to a severely blighted plant, which apparently was infected earlier and is the focus of the infection area. Usually, however, the diseased plants are more scattered and there is no clear evidence of spread from a center. In many cases only the blades of the larger leaves are involved. This indicates that the late infection was not brought about by the surface extension of the causal organism from earlier basal infections on the same plant, but by basidiospore infections. With the onset of dry weather the diseased spots on the leaves fade to a light color and become brittle. The brushing together of the leaves by the wind causes the dead areas to shatter out and leave holes that bear superficial resemblance to injury by insects or hail.

Microscopic examination of the margins of shattered-out rents in a leaf reveals *Rhizoctonia* hyphae. When a rather long humid period is followed by a short dry one, a dead central area may be surrounded by a green border on which *Rhizoctonia* threads creeping on the leaf surface are clearly visible. When a second humid period occurs, a zone or a more or less complete ring of affected tissue may appear a centimeter or more from the initial dead area, indicating a second onset of the disease. Presumably this is due to a second penetration of the leaf tissue by the fungus.

The foliage-blighting *Rhizoctonia* was found capable of causing both preemergence and postemergence damping-off of sugar-beet seedlings. In greenhouse tests damping-off has often been severe, and the fungus appears able to persist in the soil. In field plantings at Beltsville, Md., however, no serious damping-off attributable to the foliage-blighting *Rhizoctonia* has occurred. After the seedling emerges, the fungus may attack the cotyledons, the first young leaves, and the growing point, retarding growth and in some cases causing death. Attack on the growing point generally results in necrosis of the leaf tips and margins of the unfolding leaves. On further growth the injured leaves present a tipburned, blackened appearance characterized by irregularity of the leaf outline. The disease is more conspicuous on somewhat older sugar-beet plants.

## PATHOGENICITY STUDIES

### METHODS OF INOCULATION

The pathogenicity of numerous isolates of the *Rhizoctonia solani* associated with the foliage blight was tested under greenhouse conditions by inoculations of sugar beet, as well as of other plants including bean (*Phaseolus vulgaris* L.), alfalfa (*Medicago sativa* L.), Italian ryegrass (*Lolium multiflorum* Lam.; *L. italicum* A. Br.), bromegrass (*Bromus inermis* Leyss.), and potato. The pathogenic effects on foliage, on stand of seedlings, and on half-grown roots of sugar beets received most attention.

The inoculum for pathogenicity tests on sugar beets and the other species was obtained by making cultures of the various isolates on 8 ounces of sterilized sand-corn-meal medium (95:5) in 500-cc. wide-mouthed flasks. Usually a culture period of 2 weeks was used.

In the tests on sugar-beet and grass seedlings aliquots of sterilized compost soil, sufficient to fill three previously sterilized 6-inch flower-pots, were inoculated by adding an 8-ounce culture of each isolate. After a thorough mixing the soil was placed in the 6-inch pots and allowed to stand on rotating tables for 1 week before the seeds were planted. The utensils used in preparing the mixtures of soil and inoculum were disinfected between sets by wiping them with a piece of cheesecloth saturated with a 1:25 formaldehyde-water solution.

In the studies of damping-off of sugar beet 25 untreated seed balls were planted per pot. They were spaced half an inch apart and planted 1 inch deep. The seeding rates for alfalfa and grasses were comparable with those customarily used in the field. The seeds were sown broadcast and then worked into the surface soil with the wooden label placed in each pot.

The soil was inoculated in the same manner for the potato experiments. The seed pieces, cut from tubers previously disinfected for 30 minutes in a solution of mercuric chloride and water (1:1,000), were placed 4 inches below the surface of the soil at the time when the pots were being filled with the inoculated soil. The emergence of the sprouts essentially synchronized with the germination and emergence of the sugar-beet, alfalfa, and grass seedlings.

## INOCULATION OF LEAVES, PETIOLES, AND CROWNS OF SUGAR BEET

The effect of various *Rhizoctonia* isolates on sugar-beet leaves was studied in two humidity compartments of the greenhouse. These 9-by 9-foot, glass-walled compartments communicated with the main greenhouse by a glass door on one side and were equipped on the other three sides with 30-inch greenhouse benches. Devices were installed to maintain essentially 100-percent relative humidity and to control temperatures. A water line controlled by a single valve was placed 5 feet above the benches so that water might be sprayed into the air from seven evenly spaced, atomizing nozzles. A cheesecloth housing, consisting of ceiling and back drop, was supported on a frame over the benches to screen the plants on the benches from drip or direct fall of the spray. The cheesecloth cover caught surplus water and prevented excessive accumulation of water on the leaves. Without such protection inoculum placed on the leaf would have been washed off. The saturated cheesecloth also served to maintain the relative humidity at 100 percent for a considerable period after the sprays had been turned off. A thermostatically controlled unit heater, which permitted maintaining approximately 21° C. temperature at night, was installed in each compartment. The temperature in daytime was also controlled by the unit heaters, supplemented by manual regulation of individual outside ventilators on the compartments. Small quantities of inoculum were placed on the leaf blades, on the petioles, and in soil adjacent to the roots to assure the fungus reaching the growing points and petioles. *Rhizoctonia* isolates from plant parts affected with various diseases were tested (table 1).

TABLE 1.—*Pathogenicity of isolates of Rhizoctonia from foliage blight of sugar beet and from other sources, 14 days after inoculation of sugar beets*

[Each symbol based on results of 3 replications]

## ISOLATES FROM FOLIAGE BLIGHT OF SUGAR BEET

Isolate	Source of isolate	Place of collection	Year of isolation	Effect <sup>1</sup> on—		
				Leaves	Petioles	Crowns
R-254-----	Blighted leaf-----	Arlington, Va-----	1938	++	++	+
R-352-----		do-----	1940	++++	++	+
R-355-----		do-----	1940	+++	++	+
R-360-----		Doylestown, Wis-----	1940	++	++	+
R-394-----		Beltsville, Md-----	1942	++	+	+

## ISOLATES FROM CROWN AND ROOT ROT OF SUGAR BEET

R-167-----	Rotted root-----	Arlington, Va-----	1934	+	+++	+++
R-216-----		Wheatland, Wyo-----	1936	+	+++	+++

## ISOLATES FROM POTATO

R-72-----	Sclerotium on tu-	Greeley, Colo-----	1926	—	—	—
R-189-----	her.	Presque Isle, Maine-----	1934	—	—	—
R-311-----	Canker on sprout-----	Arlington, Va-----	1938	—	—	—
R-76-----	Spot on leaf-----	East Lansing, Mich-----	1929	—	—	—

<sup>1</sup>—, No tissue involvement; +, slight spotting, spots not more than 0.5 cm.; ++, spots average more than 1 cm.; +++, spots average 2 cm. or more; +++, third or half of leaf blighted. Inoculation with sterile medium resulted in no infection.

Under the favorable moisture and temperature conditions of the compartments, the strains of the foliage-blighting *Rhizoctonia* (R-254, R-352, R-355, R-360, and R-394), representing isolates obtained over a number of years and at various places, gave evidence of infection after 24 hours and the area of parenchyma involved was at least 1 cm. broad in 48 hours. In the first 4 days of this test the maximum extension of the fungus on a fully expanded leaf caused a necrotic area 8 cm. in diameter. This degree of tissue involvement was in decided contrast to that caused by the other *Rhizoctonia* strains included in the test, since there was no visual evidence of infection by these isolates even after 48 hours. Rapidity of infection and the rather extensive involvement of tissues distinguished the foliage-blighting type of *Rhizoctonia* from the root-rotting type obtained from sugar beet. The latter, however, may tardily cause slight infection; for example, after 14 days' exposure only limited spotting of the sugar-beet leaves at points of inoculation was noted (table 1). The four isolates from potato included for comparison (R-72, R-76, R-189, and R-311) were nonpathogenic to sugar-beet leaves. However, isolate R-76, obtained from a leaf spot on potato, was found in other tests to be mildly pathogenic. Inoculations of sugar-beet petioles with isolates from potato indicated that they were not able to invade such tissues.

The mycelium that grew from the inoculum of the isolates from foliage blight that was placed in the soil near the sugar-beet plants caused shallow, brown cankers on the petioles and distortion. The leaves, however, did not wilt. The young, emerging leaves at the growing point of the plant also were attacked and reduced to tip-burned stubs similar to those found in the field. In some cases the continued attack of the foliage-blighting *Rhizoctonia* caused the destruction of all the foliage. Upon removal to drier atmospheric conditions, however, many such plants produced new leaves largely free from spots.

The attack resulting from placing inoculum of the isolates from sugar-beet crown and root rot in and on the surface of the soil near the sugar-beet plants was noticeably different from that caused by the foliage-blighting isolates just described. Instead of forming shallow cankers, these isolates invaded the base of the petioles and caused them to blacken and to become so weakened that the leaves wilted and flattened down on the surface of the soil. The roots rotted at the crown, and when removed to drier atmospheric conditions such plants did not recover.

#### INOCULATION OF SUGAR-BEET SEEDLINGS

In 1941, 33 *Rhizoctonia* isolates, obtained from the foliage blight of sugar beets in different years and from several geographic locations, were tested for pathogenicity to sugar-beet seedlings. The results obtained in this test (table 2) and in many others indicate that some of the isolates that cause blighting of sugar-beet foliage are also highly pathogenic to seedlings, killing 100 percent of the inoculated plants. There is considerable variation in the pathogenicity of the several isolates; some are extremely aggressive, whereas others are only mildly pathogenic.

TABLE 2.—Pathogenicity of 83 isolates of the *Rhizoctonia* from foliage blight of sugar beet as indicated by relative stands of sugar-beet seedlings 3 weeks after planting in inoculated soil, 1941

[Each value is the average of 3 replications]

Year and place of collection and isolate	Stand as compared with that of check <sup>1</sup>	Year and place of collection and isolate	Stand as compared with that of check <sup>1</sup>	
1938				
Arlington, Va.: R-254.....	Percent 38.5	Arlington, Va.—Con. R-347.....	Percent 2.4	
R-255.....	3.8	R-348.....	.7	
R-256.....	9.6	R-349.....	0	
R-257.....	13.5	R-350.....	17.5	
R-258.....	5.5	R-351.....	.7	
R-259.....	.7	R-352.....	1.4	
R-260.....	3.8	R-353.....	0	
R-261.....	23.1	R-354.....	8.6	
R-263.....	6.2	Wasco, Minn.: R-355.....	0	
R-266.....	36.1	R-356.....	0	
R-267.....	5.5	R-357.....	0	
R-268.....	.0	R-358.....	0	
R-269.....	.7	R-359.....	0	
R-270.....	.0	Doylesboro, Wis.: R-360.....	36.8	
1940		1.4	R-361.....	67.3
R-344.....	.0	R-362.....	44.0	
R-345.....	.0			
R-346.....	.0			

<sup>1</sup> The checks in this experiment had actual stands of 38, 42, and 45 seedlings.

#### INOCULATION OF SUGAR-BEET ROOTS

Several experiments were conducted to determine the pathogenicity of the foliage blight *Rhizoctonia* to sugar-beet roots. In one such experiment three isolates representative of the foliage-blighting *Rhizoctonia* strains, three representative of the root-rotting types, and three from potato were tested for pathogenicity to sugar-beet roots. Field-grown roots of 3- to 6-cm. diameter were disinfected in a 1:1,000 solution of mercuric chloride for 30 minutes. The roots were then rinsed in distilled water and dried. After this treatment the roots were planted in 5-inch pots that had been sterilized and filled with sterilized sand. The taproots of half the collection were not injured before inoculation, and the inoculum was placed adjacent to the root approximately 1 inch below the surface of the sand. The remaining roots were injured by removing a plug about 1 cm. in diameter and 3 cm. long by means of a cork borer. The resulting hole was packed with inoculum held in place by a 5-mm. section of the plug forced into the opening. The roots were then planted. All pots were watered regularly with tap water and were kept on rotating tables to equalize temperature and light conditions. After 1 month the roots were removed and data were recorded. The existence of softened, discolored tissue more than 2 mm. from the wounded surface was taken as evidence that some invasion of the root had taken place.

The results from this experiment do not require tabulation, since they were very definite for the three types of *Rhizoctonia*. The foliage-blighting strains did not invade the uninjured taproots at all, and the extension of injury or decay at the borders of the hole made by removing a plug of tissue was small and probably not attributable to the inoculum. Similarly, the isolates from potato did not cause positive infection of either the uninjured or the injured roots. On

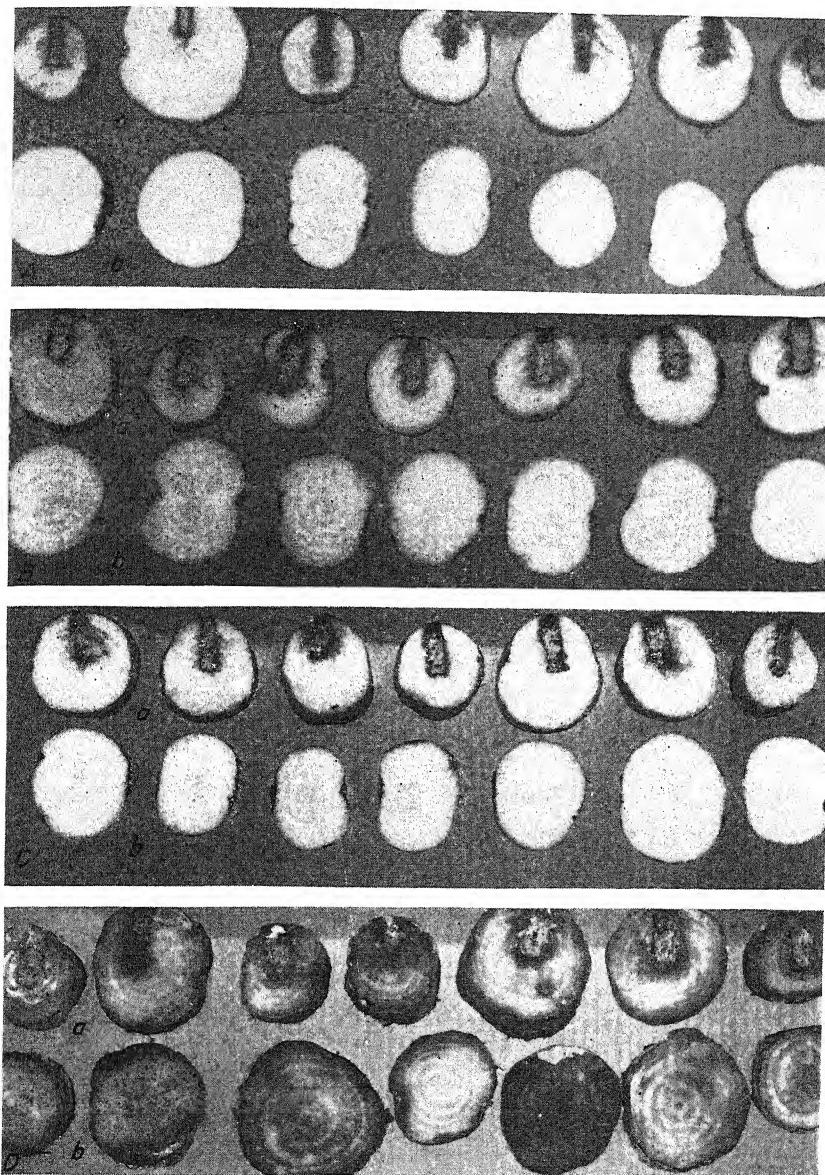


FIGURE 4.—Sections of sugar-beet roots made through the points of inoculation: *A*, No organism (check); *B*, *Rhizoctonia* isolated from sugar-beet foliage blight (R-355); *C*, *Rhizoctonia* isolated from a sclerotium on potato (R-72); *D*, *Rhizoctonia* isolated from typical sugar-beet crown and root rot (R-216). *a*, Roots injured prior to inoculation; *b*, roots not injured.

the other hand, the root-rotting types invaded the roots and caused typical rhizoctonia rot, whether the roots were wounded or not. Figure 4 illustrates the results obtained.

The pathogenicity of various *Rhizoctonia* isolates to sugar-beet roots was tested under field conditions for a number of years, and isolates of foliage-blighting *Rhizoctonia* were included from time to time. Of 53 such isolates tested, 48 were definitely nonpathogenic to sugar-beet roots. Some rotted plants were found in the plots in which the other 5 isolates were used, but these results are discounted because of occasional rotting of the adjacent check roots due to the natural occurrence of some other pathogenic *Rhizoctonia* in the field. Root rotting is, therefore, not to be expected from the *Rhizoctonia* strains that cause foliage blight, but it is not entirely precluded.

#### CROSS-INOCULATION OF VARIOUS SPECIES

The pathogenicity studies on species other than sugar beets were limited to Stringless Green Refugee bean, Grimm alfalfa, Italian ryegrass, bromegrass, and Green Mountain potato. The technique used is described on page 293. The tests consisted chiefly in a study of the pathogenic effects of the isolates from the sugar-beet foliage blight on the seedling or early growth of the various crop plants, but other *Rhizoctonia* isolates were included for comparison. The experiments on all crops were not carried on simultaneously but at intervals during the course of the work. For instance, bean and potato were included in one series of experiments and alfalfa, Italian ryegrass, and bromegrass in another. The results of these tests showed that the foliage blight *Rhizoctonia* is pathogenic to all of these crops. On bean, however, the variability among the foliage isolates is again shown; one isolate (R-394) proved nonpathogenic, another moderately pathogenic, and the other three severely pathogenic to this host

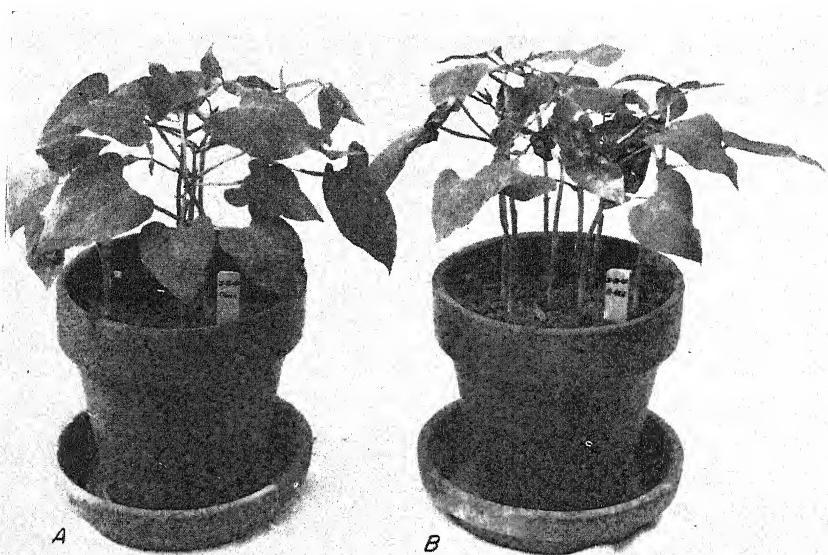


FIGURE 5.—Leaves of bean plants: A, Not inoculated (check); B, inoculated with a pure culture of *Rhizoctonia* from a sugar beet affected with foliage blight (R-422).

(table 3). On bean the cotyledons are attacked and deep cankers form on the stems. Blighting of the bean foliage also occurs when inoculated plants are kept under conditions of high relative humidity and high temperature (fig. 5). Alfalfa stands were reduced by the foliage blight *Rhizoctonia* to from 4.0 to 7.3 percent of the stand obtained from seed planted in sterilized soil. Retarded emergence and greatly reduced stands of Italian ryegrass and bromegrass resulted from inoculation with four isolates. Near the conclusion of the experiment one pot each of ryegrass and bromegrass for each of the isolates used was transferred to the humidity compartment (p. 294) and subjected to a misty spray. The foliage-blighting isolates attacked the blades of the grasses within 96 hours and caused their complete destruction in a week.

In the inoculation tests with potatoes the number of days elapsing from the date of planting to emergence of the sprouts, as well as the

TABLE 3.—*Pathogenicity of various isolates of Rhizoctonia from sugar-beet foliage blight in comparison with that of isolates from other hosts on bean, potato, alfalfa, Italian ryegrass, and bromegrass*

[Each value is the average of 3 replications]

ISOLATES FROM FOLIAGE BLIGHT OF SUGAR BEET

Isolate	Source of isolate	Place of collection	Year of isolation	Disease produced on—				
				Bean (stand as compared with that of check)	Potato <sup>1</sup>	Alfalfa seedlings (stand as compared with that of check)	Italian ryegrass <sup>1</sup>	Brome-grass <sup>1</sup>
R-254		Arlington, Va.	1938	Percent	12.5	+	-----	-----
R-352		do	1940		.0	+	-----	-----
R-355		Waseca, Minn.	1940		.0	+	-----	-----
R-360		Doylestown, Wis.	1940		50.0	+	-----	-----
R-394	Blighted leaf	Beltsville, Md.	1942	Percent	100	+	Percent	-----
R-420		do	1943				4.0	-----
R-421		do	1943				7.3	+
R-422		do	1943				5.5	+
R-423		do	1943				4.0	+

OTHER RHIZOCTONIA ISOLATES INCLUDED FOR COMPARISON

R-167	Crown rot of sugar-beet roots.	Arlington, Va.	1934	0.0	+	-----	-----	-----
R-216	do	Wheatland, Wyo.	1936	.0	+	100	-	-
R-403	do	Beltsville, Md.	1942	.0	+	-----	-----	-----
R-72	Sclerotium on potato tuber.	Greeley, Colo.	1926	100	+	-----	-----	-----
R-189	do	Presque Isle, Maine.	1934	100	-	-----	-----	-----
R-301	Canker on potato sprout.	Arlington, Va.	1938	100	-	-----	-----	-----
R-76	Spot on potato leaf.	East Lansing, Mich.	1929	.0	+	-----	-----	-----
R-40	Alfalfa roots (infected).	do	1924	-----	-----	4.0	-	-
R-291	do	Lovell, Wyo.	1938	-----	-----	1.5	-	-
R-332	do	Arlington, Va.	1934	-----	-----	.0	+	+
R-397	do	Bard, Calif.	1942	-----	-----	100	-	-

<sup>1</sup> +, Positive infection on majority of plants exposed; -, absence of infection.

height of the plants after 2 weeks of growth, afforded very effective means of appraising the pathogenic effect of the foliage blight *Rhizoctonia* strains in comparison with those of the other *Rhizoctonia* isolates. The underground parts of the plants were examined after completion of the experiment. The examination showed that the retardation of emergence of sprouts in the foliage blight series was attributable to the attack of the fungus on the eyes of the tuber seed pieces while they were sprouting. Some growth took place, but the attack continued as the sprouts were growing through the soil. In

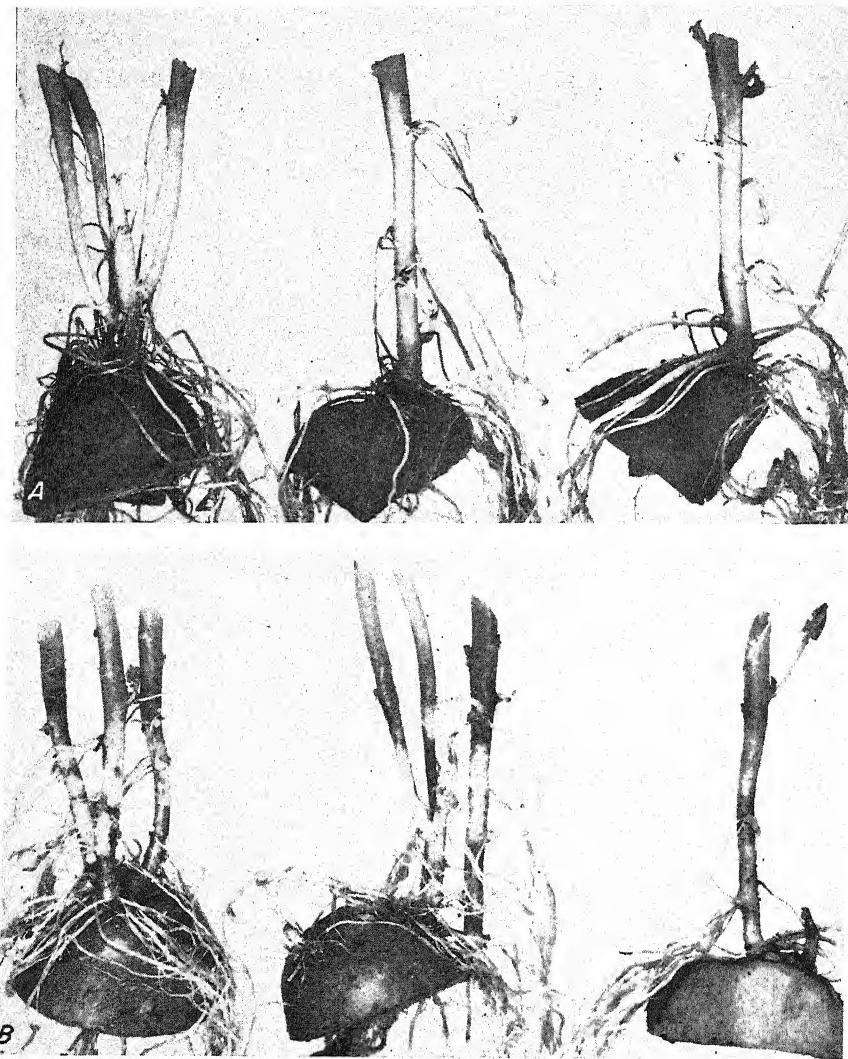


FIGURE 6.—Sprouts from potato seed pieces in soil: A, Containing sterile medium; B, inoculated with a strain of *Rhizoctonia* (R-360) isolated from sugar-beet foliage affected with blight. Note cankers on the sprouts in B.

some cases the growing point was injured to such an extent that a new leader developed from a lateral bud on a sprout and this in turn was attacked by the fungus (fig. 6). Both the foliage blight *Rhizoctonia* and the isolates from sugar-beet crown and root rot produced numerous cankers on the potato sprouts that grew in soils inoculated with them. However, the sprouts from seed pieces planted in soils that were inoculated with isolates obtained from sclerotia on potato tubers and from a canker on a potato sprout emerged as rapidly as the sprouts of tubers planted in uninoculated soil. It is of interest, however, that R-76, obtained from a spot on a potato leaf, very pronouncedly retarded top growth of potatoes. The sprouts were excessively cankered by this isolate.

The results of the pathogenicity tests show that, although the isolates from sugar-beet foliage blight fall into a general group, they exhibit a considerable range in pathogenicity (tables 1, 2, and 3).

### THE PATHOGEN

The foliage blight of sugar beet was recognized from the beginning of the experimental work as assignable to *Rhizoctonia solani*. Investigations of the *Rhizoctonia* stage were chiefly cultural.

#### CULTURAL REACTIONS OF MYCELIAL ISOLATES

From affected leaves and petioles of sugar-beet plants growing in Virginia, Maryland, Wisconsin, and Minnesota, 52 isolates were obtained. The majority were from diseased plants in experimental sugar-beet plots at Arlington, Va., or at Beltsville, Md. As was to be expected from experience with *Rhizoctonia* isolates from crown rot of sugar beets, these isolates, although causing strikingly similar symptoms on sugar-beet foliage, were variable in growth habit, rate of growth, and color when grown on potato-dextrose agar in test tubes or petri dishes.

At first mycelial growth is white, but later it ranges from light yellowish brown (10 YR 6.0/3.5) (5, 7, 17) to dusky brown (10 YR 1.8/2.3). It may be dense and largely aerial. The aerial growth tends to be fluffy because of the formation of loose aggregates of hyphae more or less sclerotial in nature. Or the mycelial growth may be confined for the most part to the surface of the medium. From a study of numerous isolates having the capacity to cause typical symptoms of foliage blight, the mycelium was found to average about  $6.4\mu$  in diameter. The fungus grows very rapidly on artificial media as well as on the natural host. At room temperature the average rate of growth of some cultures on 2-percent agar containing no added nutrients, as measured under the microscope, was approximately  $11\mu$  per minute.

The sclerotia formed by the fungus on potato-dextrose agar are similar to those of the *solani* group of *Rhizoctonia* (21). They vary from loose-textured to compact masses and from few to many. In size also they vary, some cultures producing sclerotia as large as 1 cm. in diameter.

The darkening of the substrate due to diffusion of substances from the fungus growth varies from practically none to a medium amount giving the potato-dextrose agar a color value of dark yellowish brown

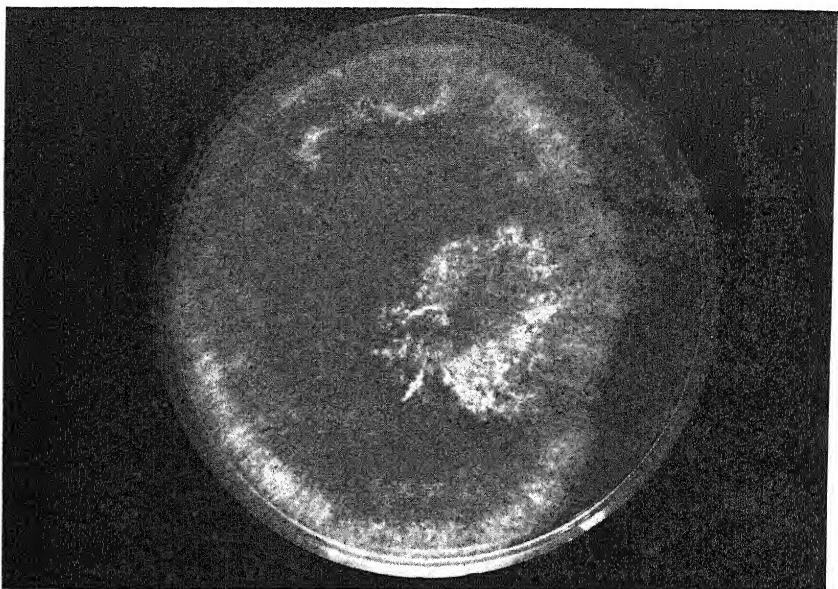


FIGURE 7.—Sector formed by a hyphal-tip isolate of the *Rhizoctonia* that causes foliage blight of sugar beets (R-361).

(0.5 YR 1.7/3.3) when viewed with transmitted light. A heavy diffusion into the substrate produces a color classified as brownish black (5 YR 0.7/0.8).

Sectors have been observed occasionally in the cultures, but these have not been studied in detail (fig. 7).

#### THE PERFECT STAGE

#### MORPHOLOGY AND SYNONYMY

The perfect stage of *Rhizoctonia solani* has been known since 1891, when Prillieux and Delacroix (18) described it as *Hypochnus solani*. About 10 years later Rolfs (20) found the perfect stage on potato stems and reported it as *Corticium vagum* Berk. and Curt. var. *solani* Burt. He trapped basidiospores by placing agar plates a short distance beneath hymenial material. These spores germinated, produced typical *Rhizoctonia* growth, and formed sclerotia (21). Since that time the relation of *Rhizoctonia* to a *Corticium* stage has been generally accepted. Although the perfect stage of *Rhizoctonia* has occasionally been collected from diseased plants or from soil or inert objects nearby, the appearance of this stage has been relatively infrequent compared with that of the asexual stage. Furthermore, it rarely has been found in ordinary cultures made from diseased plants (1, 23). Hence, it has become almost habitual to neglect the perfect stage in phytopathological studies and to minimize or disregard its function in pathogenesis. Attention of pathologists has been directed chiefly toward *Rhizoctonia* strains as pathogens, and the perfect stage has been utilized in classification of the group species *Rhizoctonia solani* and related strains.

The fungus causing foliage blight of sugar beet is distinctive among the strains of *Rhizoctonia solani* in that the basidiospore stage is commonly found in the field whenever the disease is prevalent. The hymenial stage of the foliage blight *Rhizoctonia* consists of loose wefts of mycelium that arise from vegetative hyphae through repeated right-angled branching. Globular or oblong cells much shorter than the vegetative cells are produced, and these in turn may form one or more branches. The latter may become basidia or they may continue branching. Infrequently a basidium may form directly from a vegetative cell. Four knoblike projections arise from the basidium and become tapered sterigmata. A basidiospore forms at the tip of each elongated sterigma; when the spore is mature, its longest diameter approximates the length of the sterigma. When the mature basidiospores are discharged, the sterigmata remain as sharp-pointed projections from the basidium. The basidiospores range from  $8.03\mu$  by  $4.82\mu$  to  $12.86\mu$  by  $8.03\mu$ . The average of 200 measurements of spores from field collections and of those produced by isolates under humid conditions in the greenhouse was  $9.92\mu$  by  $6.97\mu$ ; no collection or isolate deviated enough from this mean to warrant particular attention.

Recently Rogers (19) proposed the name *Pellicularia filamentosa* (Pat.) Rogers to include the organisms previously classed (20) as *Corticium vagum* var. *solani*. The size and other characters of the spores would permit the classification of the fungus causing foliage blight as *P. filamentosa*.

#### METHODS FOR OBTAINING THE PERFECT STAGE OF VARIOUS STRAINS OF RHIZOCTONIA

Britton-Jones (2) and Müller (16) observed the formation of the perfect stage of *Rhizoctonia* strains after infection of the hosts. Müller used single-basidiospore cultures for making inoculations. In general, occurrence of the perfect stage was found to be sporadic and not subject to control.

A number of isolates of the foliage blight fungus produced the perfect stage on the leaves and petioles of sugar-beet plants kept under favorable conditions in the greenhouse (approximately 100 percent relative humidity and temperatures between  $21^{\circ}$  and  $25^{\circ}$  C.). For comparison a number of other *Rhizoctonia* isolates from various hosts were tested on sugar-beet roots and leaves to determine whether any of them could be induced to form the perfect stage. Sugar-beet plants growing in sterilized soil in pots and having roots approximately 2 cm. in diameter were placed in the humidity compartments described on page 294. The roots and leaves of the plants were inoculated with vigorously growing cultures of a number of hyphal-tip isolates of *Rhizoctonia*. After approximately 2 weeks the hymenial stage was detected on the petioles and leaves of the sugar-beet plants that had been inoculated with *Rhizoctonia* isolates from alfalfa, black locust (*Robinia pseudoacacia* L.), cotton (*Gossypium hirsutum* L.), horseradish (*Armoracia rusticana* Gaertn., Mey., and Scherb.), rubber (*Hevea brasiliensis* (H. B. K.) Muell. Arg.), sugar beet, stock (*Matthiola incana* R. Br.), strawberry (*Fragaria virginiana* Duchesne), sweet-clover (*Melilotus alba* Desr.), and tobacco (*Nicotiana tabacum* L.).

Of this work only that concerning *Rhizoctonia* isolates attacking cotton and rubber has been reported (11, 12). The study indicates that many hyphal-tip isolates of *Rhizoctonia* can be induced to form the perfect stage on living plants under more or less controlled conditions. Of the factors involved, moisture and temperature seem to be very important.

Müller (15) was the first investigator to report even meager success in obtaining the perfect stage of *Rhizoctonia solani* on artificial media. He grew the fungus on a rich medium for 17 days and then transferred portions of the mycelial growth to wet filter paper in small flasks. Basidiospores formed on the filter paper in two of the cultures. Müller was not able, however, to duplicate his results. Ullstrup (25) observed the formation of the perfect stage of *R. solani* as a result of plating sections of affected cotton seedlings on tap-water agar. He made transfers of mycelial, monosporous, and multisporous material to tap-water agar containing pieces of healthy cotton seedlings that had been surface-sterilized. Only the transfers of multisporous material again produced the perfect stage. Exner and Chilton (3) obtained the perfect stage of *R. solani* by growing certain tissue cultures on potato-dextrose broth, rinsing them in distilled water, and then transferring them to flasks containing rooted cuttings of alligatorweed (*Alternanthera philoxeroides* (Mart.) Griseb.). The perfect stage formed on the stems of the alligatorweed. Exner and Chilton reported failure under these conditions to obtain the perfect stage from single-basidiospore cultures.

Kotila (9), on the other hand, found that the single-basidiospore isolates of *Corticium praticola* formed the perfect stage on the artificial media used without the addition of plant tissue. The mass culture from which these single-spore cultures were obtained formed the perfect stage on artificial culture media and on alfalfa when it was placed under suitable environmental conditions. Since this report was made, six additional hyphal-tip isolates of *Rhizoctonia solani* from alfalfa and sugar beet that formed the perfect stage in petri dishes on 2-percent distilled-water agar have been obtained. It has also been possible to induce formation of the perfect stage by some *Rhizoctonia* cultures on the walls of flasks containing small quantities of dilute Coons' synthetic solution by following the Klebs' (8) principle of first obtaining vigorous vegetative growth of the fungus and then transferring to a less nutritive substrate.

#### BEHAVIOR OF BASIDIOSPORE CULTURES OF FOLIAGE BLIGHT FUNGUS

During the course of the investigations of rhizoctonia foliage blight of sugar beet a study was made of a number of single-basidiospore isolates obtained from *Pellicularia filamentosa*. The isolations were made by distributing with a capillary pipette (6) highly dilute suspensions of basidiospores on the surface of hardened plain agar in petri dishes. Forty-eight hours later the plates were examined and single basidiospores that had germinated were located and marked by means of a fine-wire ring attached to an objective of the microscope. The marked spores were transferred to hardened plain agar in other petri dishes and examined under the microscope again to be certain that only 1 spore was present; then the spore was transferred to a test

tube of slanted potato-dextrose agar. Fifty-one germinated single basidiospores from the perfect stage produced on a sugar-beet plant inoculated with isolate R-423, a typical foliage blight *Rhizoctonia*, were transplanted in this manner. Of these cultures, 38 failed to continue growth. The other 13 grew, and macroscopic differences in growth habit soon became evident. Their characteristics were also different from those of isolate R-423. Transfers were made to hardened potato-dextrose agar in petri dishes, and after a week transfers of equal portions of each culture were made to a second series of petri dishes containing the same kind of medium. Three transfers were made from each culture. The growth produced in each of the 3 petri dishes from a given culture was strikingly similar, but there were definite differences in growth habit among sets that traced back to different basidiospores.

The cultures obtained from single basidiospores were somewhat slow in starting to grow, but once established they grew rapidly and apparently almost as vigorously as those from mycelial transfers. In general appearance a series of basidiospore cultures was indistinguishable from a series of mycelial cultures of *Rhizoctonia*. The different basidiospore isolates showed ranges in color, in degrees of discoloration of medium, of zonation, and of sclerotial formation, and in other characteristics that paralleled those usually noticed in *Rhizoctonia* cultures (figs. 8 and 9). Almost any characteristic selected from mycelial cultures could be matched by a similar one in one basidiospore culture or another. In other words, the basidiospore cultures exhibited about the same range of growth characters and had about the same general appearance in culture as would be found in a series of mycelial isolates.

#### INFECTION BY BASIDIOSPORES OF FOLIAGE BLIGHT FUNGUS

The important role that basidiospores may play in the dissemination of the rhizoctonia foliage blight of sugar beets, referred to in the discussion of the probable course of the disease in the field (p. 291), was clearly indicated by the results of several experiments conducted in the two humidity compartments in a large unit of the greenhouse described on page 294.

The design of the experiments was such that healthy plants could be exposed to air-borne basidiospores of the foliage blight *Rhizoctonia*, but the plants were so placed that infection by direct contact was avoided. Greenhouse-grown sugar-beet seedlings 3 to 4 inches tall were used as trap plants for the basidiospores. In 1 test 22 plants, growing in 6-inch pots of sterilized soil and designated as group 1, were placed with uniform spacings on the side benches of each of the 2 compartments. One week later 26 additional sugar-beet seedlings, growing in 3-inch pots and designated as group 2, were interspersed in each compartment among those of the first group. During the first week of the experiment the basidiospore source was a few leaves which had been collected in a sugar-beet field at Beltsville, Md., and on which the fungus was fruiting. The leaves, with the basidial-stage surface faced downward, were laid on hardware-cloth screens 18 inches above the plants, 1 being placed over each bench in 1 of the compartments. This basidiospore source was augmented later by 2 sugar-beet plants

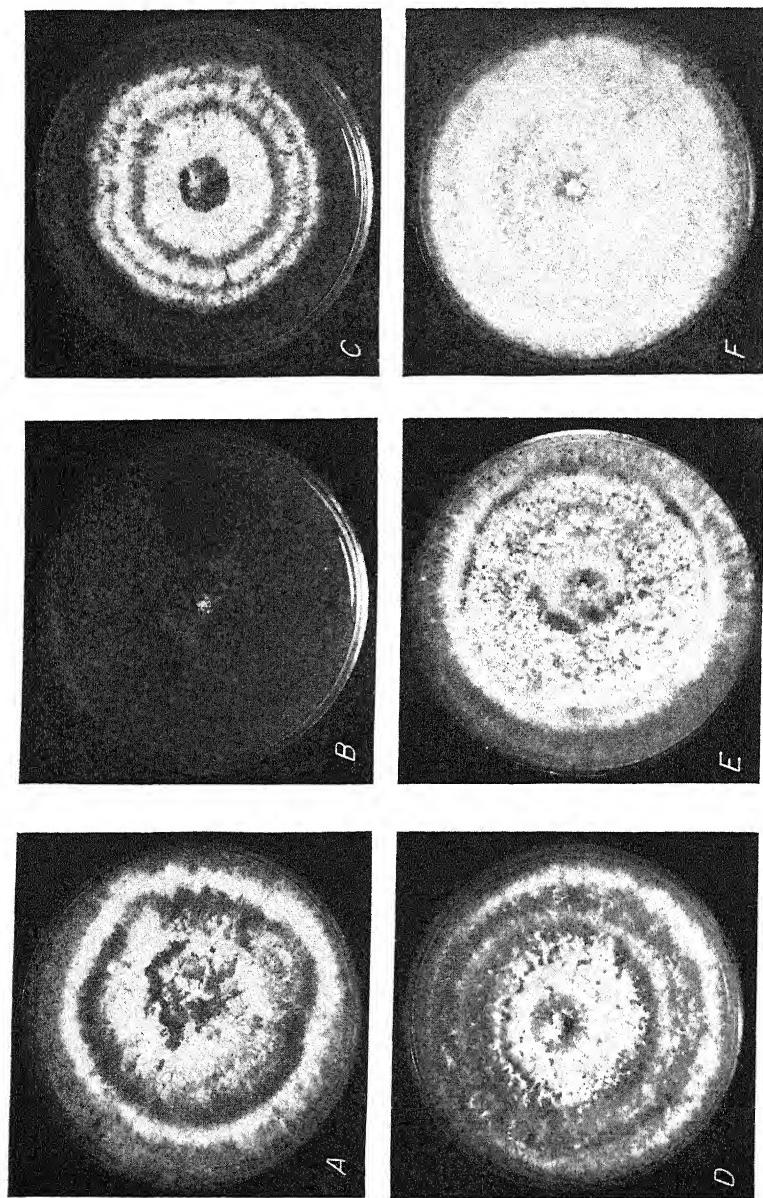


FIGURE 8.—One-week-old cultures of *Pellicularia filamentosa* on potato-dextrose agar as viewed from above: *A*, Parent mycelial culture; *B-F*, single-basidiospore cultures.

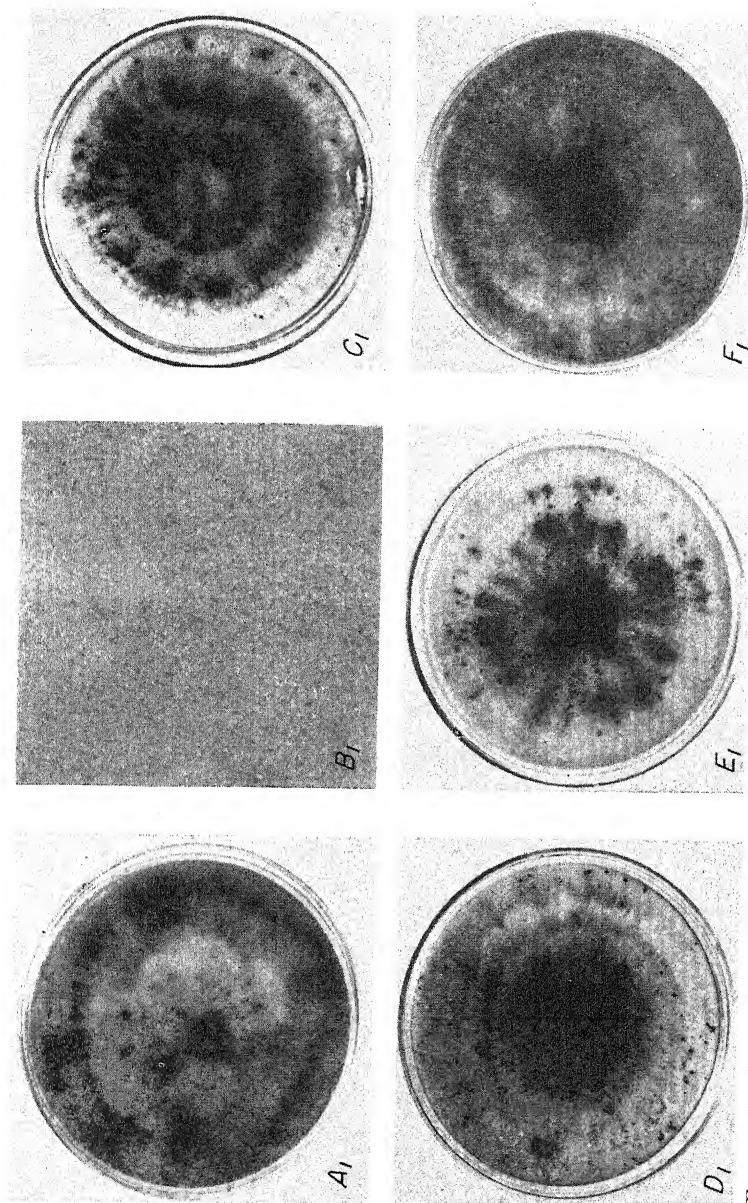


FIGURE 9.—One-month-old cultures of *Pellicularia flamencosa* as viewed through bottom of petri dish; A<sub>1</sub>, Patent mycelial culture; C<sub>1</sub>-F<sub>1</sub>, single-basidiospore cultures. In this figure C<sub>1</sub>-F<sub>1</sub>, respectively, correspond to C-F in figure 8; the culture shown in B of that figure was lost.

on which the perfect stage had formed in a previous experiment. One of the plants was placed at the same level as the screens, and the other was placed on the bench 12 inches from the nearest healthy plant. In each compartment the sprays were kept on continuously for 120 hours and then turned off and on alternately to keep the humidity high.

Ten days after the start of the experiment 16 of the group 1 plants in compartment 1 showed infection. Initial infection from basidiospores appeared as minute, sunken, water-soaked, round or irregular areas. After the affected areas had reached 5 mm. in diameter, the rate of involvement of the leaf blade was greatly increased (fig. 10).

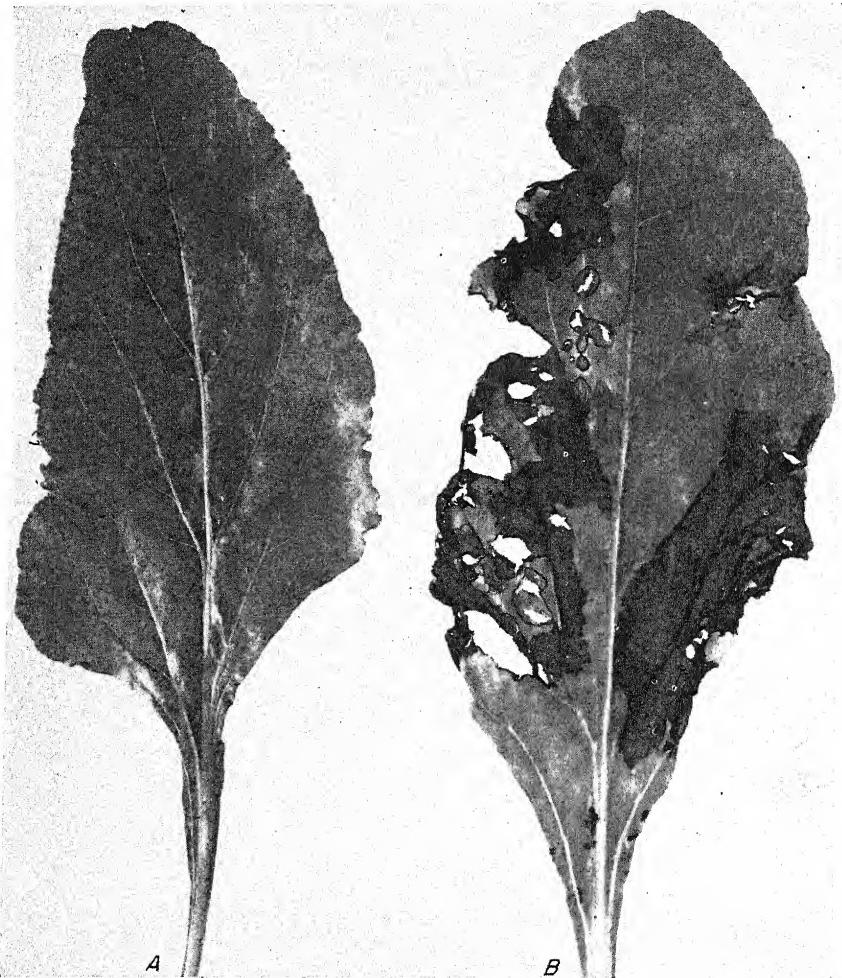


FIGURE 10.—*A*, Leaf from a sugar-beet plant grown for 14 days without inoculation in a humidity compartment in the greenhouse; *B*, leaf from a plant in a similar compartment exposed to air-borne basidiospores of *Pellicularia filamentosa*.

Microscopic examination of a number of the areas showed *Rhizoctonia* mycelium to be present. Fourteen days after the beginning of the test the 22 plants were examined in detail and the number of leaves affected and the number of distinctly separated infections brought about by the basidiospores were noted (table 4). The 22 plants in compartment 2 were all free from injury.

The final records on the experiment were taken after the group 1 plants in compartment 1 had been exposed to basidiospore infection for 24 days and the group 2 plants in the same compartment for 17 days. At this time 90.9 percent of the plants in group 1 and 76.9 percent of those in group 2 showed one or more infected areas.

TABLE 4.—*Artificial infection of sugar-beet leaves by air-borne basidiospores of *Pellicularia filamentosa* after 14 days in a humidity compartment in a greenhouse*

Plant and compartment	Leaves infected	Areas infected	Plant and compartment	Leaves infected	Areas infected
Compartment 1:			Compartment 2:		
1.....	0	0	23.....	0	0
2.....	4	30	24.....	0	0
3.....	1	2	25.....	0	0
4.....	0	0	26.....	0	0
5.....	0	0	27.....	0	0
6.....	2	16	28.....	0	0
7.....	2	15	29.....	0	0
8.....	2	23	30.....	0	0
9.....	2	30	31.....	0	0
10.....	4	47	32.....	0	0
11.....	2	17	33.....	0	0
12.....	2	17	34.....	0	0
13.....	2	4	35.....	0	0
14.....	1	3	36.....	0	0
15.....	2	15	37.....	0	0
16.....	2	19	38.....	0	0
17.....	2	20	39.....	0	0
18.....	2	17	40.....	0	0
19.....	2	26	41.....	0	0
20.....	3	20	42.....	0	0
21.....	2	20	43.....	0	0
22.....	3	10	44.....	0	0
Total.....	42	351	Total.....	0	0

Compartment 2, which was adjacent to compartment 1 and exposed to similar environmental conditions, contained the same number of plants, but the plants were not exposed to an immediate source of basidiospores. None of the plants of group 2 became infected, and all except one of group 1 remained free from the disease. The one infected plant had a small diseased area on one leaf, and the causal organism when isolated and tested was found to be typical of the foliage blight *Rhizoctonia*. The infection is believed to have resulted from chance air dissemination of a basidiospore from compartment 1.

The results obtained in these experiments are very striking and confirm the observations made in the field which indicated that the spread of rhizoctonia foliage blight of sugar beet is brought about principally through the dissemination of basidiospores.

The pathogenicity of the 13 single-basidiospore cultures described on page 305 was determined by inoculating sterilized soil and planting untreated sugar-beet seed balls in the manner previously described and by inoculating leaves of older sugar-beet plants kept under high-humidity and high-temperature conditions. Differences in pathogenicity were noticeable in both tests, but the counts of seedling

stands afford the better numerical measurement of these differences (table 5). The single-spore isolates 3 and 5, which traced to R-423 and had the slowest rates of growth on potato-dextrose agar, were the least pathogenic of the single-spore isolates; they caused the least damping-off of sugar-beet seedlings and the least foliage infection on older plants. The other single-basidiospore cultures reproduced typical foliage blight.

TABLE 5.—*Pathogenicity of parent and single-basidiospore cultures of Pellicularia filamentosa as indicated by relative stands of sugar-beet seedlings 3 weeks after the planting of 25 seed balls in inoculated, sterilized compost soil*

[Each value is the average of 3 replications]

Isolate	Stand as compared with that of check		Isolate	Stand as compared with that of check	
	Test 1 <sup>1</sup>	Test 2 <sup>2</sup>		Test 1 <sup>1</sup>	Test 2 <sup>2</sup>
R-423 (parent source).....	Percent 7.7	Percent 19.1	S-17.....	Percent 25.8	Percent 27.3
S-3.....	96.4	100.9	S-27.....	29.2	24.2
S-5.....	71.6	62.7	S-33.....	.0	.9
S-6.....	18.1	20.7	S-38.....	13.7	17.3
S-7.....	7.0	7.0	S-43.....	7.7	16.1
S-11.....	15.5	23.3	S-44.....	3.4	14.2
S-13.....	44.7	38.5	S-47.....	10.3	12.1

<sup>1</sup> An average of 38.7 seedlings in the check.

<sup>2</sup> An average of 33.0 seedlings in the check.

## DISCUSSION

In this paper a new type of disease of sugar beet assignable to *Rhizoctonia solani* is reported. In addition to crown and root rot and dry rot canker previously recognized as forms of *Rhizoctonia* attack, a foliage blight with complete absence of root complications is now known. The perfect stage of the sugar-beet foliage-blighting *Rhizoctonia* occurs under the warm, humid conditions necessary for maximum disease manifestation on sugar-beet leaves. The size of basidiospores and other characters of the causal fungus permit its assignment to *Pellicularia filamentosa*. The chief emphasis in the investigations reported was placed on utilizing information obtained from basidiospore cultures to throw light on the well-known variability exhibited by *Rhizoctonia* isolates.

Previous studies showed that isolates of *Rhizoctonia* were extremely variable in pathogenicity and that there may be some trends toward group reactions. LeClerg (13) tested 116 isolates obtained chiefly from sclerotia formed on potato tubers and from the lesions on stems of older potato plants. Among these isolates he did not find any that were pathogenic to half-grown or mature roots of sugar beet. On the other hand, *Rhizoctonia* isolates obtained from sugar beet were pathogenic to potato.

More recently Houston (4) reviewed previous work and made additional studies on 260 isolates of *Rhizoctonia* (*Corticium solani*) from 15 different crop plants. He classified the isolates into cultural types. Differences in growth habit on nutrient media such as presence or absence of stroma, nature, size, and abundance of sclerotia, and absence or presence of diffusible substances that darken the medium were criteria for differentiation. Although admitting intergrades,

Houston stated that most isolates could readily be assigned to their appropriate types. Type A was obtained from many hosts and was a polyphagous type, whereas types B and C were highly specific as to hosts. Type C was essentially nonpathogenic to sugar-beet roots. Houston stated that the variation in pathogenicity among isolates was much greater when the isolates were grouped according to their original hosts than when they were grouped on the basis of cultural type. Houston's tabular data, as the author himself pointed out, revealed a number of contradictions in the proposed classification scheme as it related to pathogenicity.

In the present study it was found that the *Rhizoctonia* isolates obtained from the foliage blight of sugar beet constitute a recognizable group. This group is distinct from the isolates previously obtained from sugar beet and other hosts in that a new and previously unrecognized disease condition in sugar beets is produced. If Houston's criteria for growth habit are applied, the isolates would fall more or less into types A, B, and C; but the absence of capacity to cause root rot of sugar beet would seem to correspond only to type C of Houston's classification. Furthermore, the isolates obtained from single basidiospores of this group also showed such a great diversity of growth habit that they could be distributed among Houston's types A, B, and C. These single-basidiospore cultures also manifested great variability in pathogenicity to sugar-beet seedlings and to sugar-beet foliage.

A logical explanation for the diversity that has so commonly been found among *Rhizoctonia* isolates is afforded by the study of these single-basidiospore cultures, presumptively haploid in character. The results of the studies of the basidiospore cultures also explain why attempts to classify asexual forms on the basis of cultural characters or pathogenicity tests must be unsatisfactory.

It is recognized that the heritable factors in fungi, as in other plants, are transmitted through the nuclei. Literature on the subject was summarized by Lindegren (14), who suggested a rational explanation of certain phenomena on the basis of nuclear behavior. As a result of preliminary studies Exner and Chilton (3) stated that the fusion nucleus of *Rhizoctonia solani* is heterozygous. Müller (16) offered evidence that the mycelial cells of *Rhizoctonia* are multinucleate. When the reproductive stage is induced, the hymenial cells become binucleate and maintain this condition throughout hymenial growth. Fusion of the two nuclei takes place in the basidium and is followed by meiosis, after which each of the four daughter nuclei finds its way through a sterigma into a basidiospore. This is the general pattern for the Hymenomycetes and each nucleus carries a haploid complement of heritable factors. Skolko (24) studied the nuclear behavior in a closely related form, *Aleurodiscus canadensis* Skolko. The evidence of homothallism in spores produced on two-spored basidia and in some spores produced on three-spored basidia indicates that if four-spored basidia develop, the basidiospores on them are normally haploid.

The great divergence in growth characters and the variability in pathogenicity found among the single-basidiospore cultures tracing to individual isolates of *Pellicularia filamentosa* may be attributed to gene segregations. These take place in the basidium as a result of the reduction division that follows sexual fusion of nuclei unlike in

genic make-up. The studies of these single-basidiospore isolates indicate that *Rhizoctonia* mycelia as commonly found in nature are not homokaryotic but are heterokaryotic; that is, they are composites insofar as nuclear complements are concerned. A study of the asexual stage is, therefore, a phenotypic study interpretable only by reference to the genotypes.

It is to be noted that growth characters and the pathogenicity reactions of both basidiospore isolates and mycelial isolates are essentially parallel in character. Hence, it is concluded that growth characters, pathogenicity, and other reactions of an individual *Rhizoctonia* isolate obtained by culturing a single-hypha cell are expressions of particular assortments of genes from the nuclei that happen to be present in the cell. The reactions shown by a given culture represent the equilibrium achieved. The great multiplicity of strains among *Rhizoctonia* isolates is, therefore, related to the heterokaryotic nature of the mycelium and to the nuclear and genic assortments possible.

#### SUMMARY

The blight of sugar-beet foliage described in this paper has been found in Virginia, Maryland, Michigan, Illinois, Wisconsin, Minnesota, and Colorado. The conditions for its maximum development include high humidity and temperatures between 21° and 25° C. The causal organism is a type of *Rhizoctonia solani*. It attacks the small, unfolding leaves of the sugar beet and may reduce them almost to stubs. It causes cankers on petioles and attacks the blades of larger leaves. This *Rhizoctonia*, which is primarily a pathogen affecting sugar-beet foliage, does not rot half-mature or older roots. It may cause both preemergence and postemergence damping-off of sugar-beet seedlings. It is pathogenic to bean, alfalfa, bromegrass, Italian ryegrass, and potato.

The various mycelial isolates exhibited a range in growth habit, color, and rate of growth on artificial media; they varied also in pathogenicity.

The perfect stage of this *Rhizoctonia* occurs on leaf blades and petioles of sugar-beet plants. The size of the basidiospore and other characters permit its classification as *Pellicularia filamentosa*. The perfect stage was induced by growing inoculated sugar-beet plants in greenhouse compartments in which the relative humidity was kept as near to 100 percent as possible and temperatures were held between 21° and 25° C.

Field observations indicated that basidiospores play an important role in dissemination of the fungus. Experiments conducted under controlled conditions in the greenhouse confirmed these observations.

The single-basidiospore isolates were found to have cultural characters distinctly different from those of the mycelial isolate from which they were obtained. They also differed among themselves in growth habit. They varied greatly in pathogenicity to sugar-beet seedlings, infection ranging from 100 percent to almost none; on sugar-beet foliage infection ranged from mild to severe. The variations displayed by the single-basidiospore cultures are interpreted as the result of segregation of heritable characters in these haploids. The nuclear

or genic assortments that make up the heterokaryotic mycelium of the *Rhizoctonia* strains found in nature explain the multiplicity of strains that occurs within this group.

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# EFFECT OF SITE ON GROWTH OF SHIPMAST LOCUST<sup>1</sup>

By HENRY HOPP, *Soil Conservationist*, and SAMUEL GROBER, formerly Agent,  
*Erosion Control Practices Division, Soil Conservation Service, United States  
Department of Agriculture*<sup>2</sup>

## INTRODUCTION

Shipmast locust (*Robinia pseudoacacia L.* var. *rectissima* Raber) is one of several newly selected forms of black locust that have above-average growth characteristics. A number of studies on shipment locust have been published (4, 5, 7, 8, 9, 10, 13, 14, 15, 18).<sup>3</sup> These have shown that on suitable sites, shipmast locust is straighter, more erect, and narrower crowned than the common black locust. The heartwood is definitely more durable and the trees may be more resistant to borer injury. However, observations on Long Island, N. Y., the principal area where old stands now occur, indicate that shipmast locust is particularly sensitive to site conditions. On favorable sites, magnificent stands of tall, well-formed trees occur, far surpassing in quality the common black locust. On other sites, growth of the shipmast locust is apparently much poorer than that of the common black locust.

In view of the degree to which the growth form of shipmast locust varies according to environmental conditions, its superiority over common black locust depends on the selection of sites to which it is adapted. Hence, planters cannot expect to obtain the advantages of the superior genetic characters of shipmast locust unless they have sufficient knowledge to choose satisfactory sites. Data relative to site relationships in established stands of shipmast locust on Long Island are given in the following study. These stands are the best source of information available at present on the site requirements of this variety.

## METHODS

Eighty shipmast locust stands over the geographic range of the variety on Long Island were chosen for the study. The stands were so selected as to cover a wide variation in site condition, growth rate, and age. All the stands were second growth from old plantations on farms and estates.

In each of the stands usually five trees were selected in the immediate vicinity of the point chosen for soil observations. Except for the avoidance of decadent and badly damaged individuals, the trees to be measured in each stand were selected at random in respect to form,

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<sup>3</sup> Italic numbers in parentheses refer to Literature Cited, p. 327.

age, and growth rate. For each of the sample trees, the total age, height, and diameter breast high were determined.

The relative growth rate for each tree was indexed by means of a site-index chart (fig. 1) which was constructed according to the method

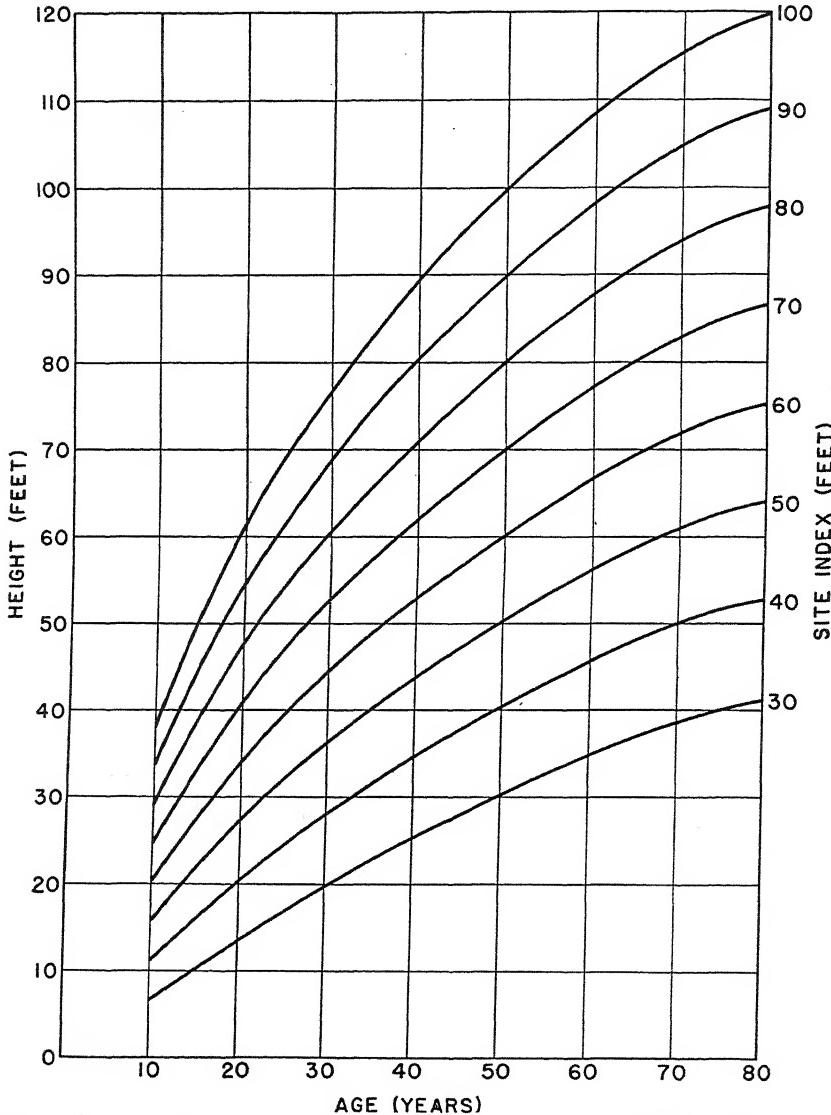


FIGURE 1.—Site-index chart for shipmast locust on Long Island. Basis: 397 shipmast locust trees, 7 to 78 years old.

described by Bruce and Schumacher (3, pp. 318-322). The site indexes for the trees in each stand were averaged to obtain the site index of the stand.

The site factors included in this study are listed in the first column of table 1.

The depth to water table was measured by three methods:

(1) When a dug well was present, the depth to the water table was measured with a weighted steel tape.

(2) When exposed surface water was present, its elevation in relation to the station was measured with a hand level and tape.

(3) When neither a dug well nor exposed water was present in the immediate vicinity, the water table was determined from the published water table surveys of the State of New York (17) and unpublished data made available by the Department of Water Supply, Gas and Electricity of New York City. These surveys were used only for stations located in areas where the permanent water table was close to the surface and the geological structure of the subsoil indicated that perched water tables were absent.

Water-table data were available for 56 of the 80 stands. The soil examination was made in a pit dug to a depth of 3 feet, from which samples were taken in each horizon for physical and chemical analyses. The standard methods of physical analysis and rapid tests (19) for chemical analysis were used.

In each stand a one-tenth-acre plot was laid out and a complete tally was made of all trees. Undergrowth and ground cover on the plots were recorded by species and the approximate percentage of the area occupied by each species was estimated by eye.

## RESULTS

### SITE FACTORS AFFECTING GROWTH RATE OF SHIPMAST LOCUST

The average site index for 56 stands of shipmast locust was 66 feet. This means that on the average, shipmast locust had been growing at the rate of 66 feet in 50 years. The slowest-growing stand showed a site index of 47 feet and the fastest-growing stand 88 feet. The

TABLE 1.—*Statistical evaluation of 39 individual site factors for indexing growth rate of 56 shipmast locust stands*

Site character	Range	Variance ratio	Site index means or regressions for significant site factors
Elevation above sea level <sup>1</sup>	0-250 feet	0.06	
Exposure <sup>1</sup>	{ Cool (N., NE., E., NW.) Warm (S.E., S., SW., W., flat)	{ 5.42* 5.47*	{ Cool=71.0. Warm=64.5. 64.0+ .27×slope percent.
Slope <sup>1</sup>	0-35 percent		
Topography <sup>1</sup>	Hillsides—17 stands; flat terrain—39 stands	27.60**	Hillsides=74.0. Flat terrain=62.5.
Depth of water table <sup>1</sup>	4-135 feet	1.68	
Soil series <sup>2</sup>	{ Plymouth Haven Sassafras Dukes	3.49*	{ Plymouth=70.5. Haven=67.2. Sassafras=62.3. Dukes=60.4.
Depth of A horizon <sup>1</sup>	1-7 inches	.69	
Depth of B horizon <sup>1</sup>	5-24 inches	.06	
Depth to C horizon <sup>1</sup>	7-31 inches	.21	
Sand, B horizon <sup>3</sup>	41-95 percent	2.02	
Sand, C horizon <sup>3</sup>	35-97 percent	2.80	
Clay, B horizon <sup>3</sup>	3-16 percent	.94	
Clay, C horizon <sup>3</sup>	3-28 percent	.43	
Pore space, B horizon <sup>3</sup>	36-66 percent	3.42	
Pore space, C horizon	28-57 percent	.13	
Water-holding capacity, B horizon <sup>3</sup>	16-92 percent	.13	
Water-holding capacity, C horizon <sup>3</sup>	13-78 percent	1.72	

See footnotes at end of table.

TABLE I.—*Statistical evaluation of 39 individual site factors for indexing growth rate of 56 shipmast locust stands—Continued*

Site character	Range	Variance ratio	Site index means or regressions for significant site factors
Chemicals in B horizon: <sup>3</sup>			
Organic matter	0.3-5.1 percent	.12	
Hydrogen-ion concentration	4.0-7.1 pH	.42	
Calcium	0-4,000 pounds per acre	.12	
Magnesium	0-200 pounds per acre	.48	
Aluminum	0-500 pounds per acre	.45	
Iron (ferric)	0-300 pounds per acre	0	
Nitrogen (nitrate)	0-100 pounds per acre	.07	
Nitrogen (ammonia)	0-25 pounds per acre	.07	
Phosphorus	0-50 pounds per acre	1.32	
Potassium	0-700 pounds per acre	.05	
Manganese	0-50 pounds per acre	1.64	
Chemicals in C horizon: <sup>3</sup>			
Organic matter	0.0-2.8 percent	.19	
Hydrogen-ion concentration	4.3-7.1 pH	.18	
Calcium	0-3,000 pounds per acre	.23	
Magnesium	0-200 pounds per acre	.25	
Aluminum	25-500 pounds per acre	.69	
Iron (ferric)	0-100 pounds per acre	.61	
Nitrogen (nitrate)	0-100 pounds per acre	1.64	
Nitrogen (ammonia)	0-15 pounds per acre	.37	
Phosphorus	0-50 pounds per acre	4.10*	67.4-0.28 pounds per acre.
Potassium	0-500 pounds per acre	.77	
Manganese	0-50 pounds per acre	0	

\*Significant at 5 percent level.

\*\*Significant at 1 percent level.

<sup>1</sup> 56 stands. Variance ratio based on 1 and 54 degrees of freedom.<sup>2</sup> 56 stands. Variance ratio based on 3 and 52 degrees of freedom.<sup>3</sup> 55 stands. Variance ratio based on 1 and 53 degrees of freedom.

effectiveness of 39 different site factors for explaining this variation in growth rate is summarized in table 1. The second column shows the range of variation for each factor. The range is important for indicating the possible application of the results to areas outside Long Island.

The variance ratio, shown in column 3, is the customary significance test used in statistical analysis (16). The variance ratios are found in the following manner. First, the total variation as represented by the mean site index of 66 feet and the range of 47 feet to 88 feet is expressed in terms of variance by conventional methods of calculation. The total variation is then divided into that part which is explained by the site factor and remainder, which is that part unexplained by the site factor. The ratio of these two parts is the variance ratio. A large variance ratio indicates that the site factor explains a large part of the site-index variation among stands. Variance ratios that are statistically significant are marked with asterisks in table 1.

The means or regressions for the five factors that were significantly associated with variations in shipmast locust site index are shown in column 4. While these relationships were significant, they were somewhat deceptive when considered without regard to one another. Because of interrelationships among them, several appeared to be more important than they actually were, while others that actually were important did not show a significant variance ratio when considered individually. Attention was therefore given to various combinations of site factors. As a result of this examination, the site classification shown in figure 2 was devised. This classification accounted for all

the significant relationships between growth rate and the site factors listed in table 1.

Figure 2 is used in the following manner. First, the site is classified into either of two topography groups: hillsides or flat terrain. By hillside stands is meant stands located on sloping land where a drainage area lies above the site. Stands in this group were fed by supplementary drainage water as evidenced by the frequent occurrence of springs and seep spots. By stands on flat terrain is meant stands located on either level, undulating, or sloping land where no drainage area lies above the site. In general, stands on hillsides showed markedly better growth than those on flat terrain.

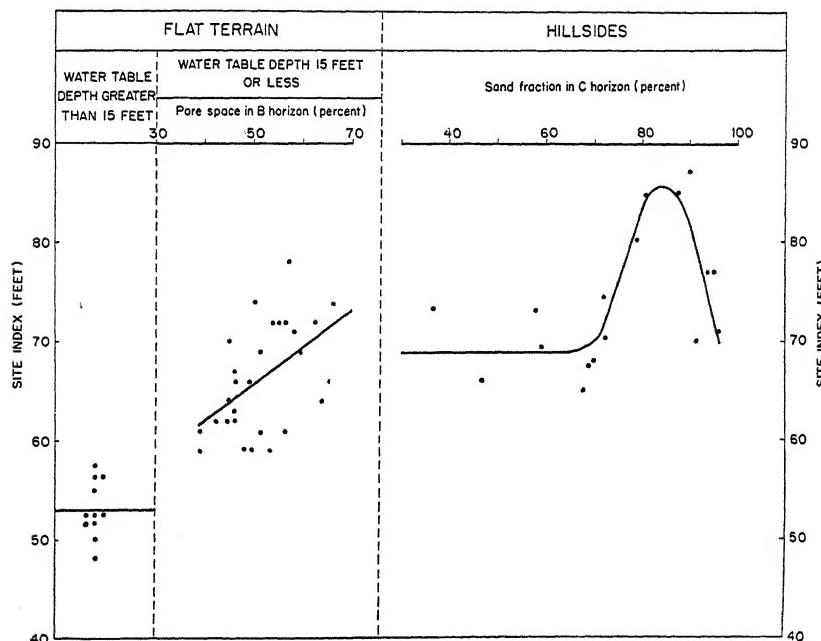


FIGURE 2.—Site classification for shipmast locust on Long Island. The plotted points are data from 56 measured stands.

Hillside stands are further classified according to the percentage of sand in the C horizon. Growth rate was more rapid on sites where the sand concentration of the C horizon was between 70 and 90 percent than on sites where the soil contained a greater proportion of finer particles or where the soil was extremely sandy.

Stands on flat terrain are next classified into two groups according to depth to a water table. On sites where the water table was deeper than 15 feet (16 to 135 feet in these samples), the average site index was 53.6 feet. On flat terrain where the water table was 15 feet or less (4 to 15 feet in these samples) the growth rate was more rapid. Among these latter stands the site index varied according to the pore space in the B horizon, the growth rate being better on the more porous soils.

The site classification of figure 2 was more effective for explaining variations in growth rate than any other set of factors that could be compounded from the 39 individual factors. The extent to which growth rate variations were accounted for is shown by the analysis of variance of table 2. The total variation, represented by a sum of squares of 4,596, is segregated into one part, 902, not accounted for by the classification. The variance of this part is 19.2. The remainder of the sum of squares, amounting to 3,694, is the part explained by the classification. Thus, the classification accounts for  $3694/4596$ , or roughly 80 percent of the total variation among stands. The relative importance of the 4 factors used in the classification is shown by the further analysis of the sum of squares. Referring to the classification of figure 2, the variance accounted for by topography was 1,571, by water table depth 1,341, by texture 114, and by pore space 213. All these values are highly significant in comparison with 19.2, the remainder variance.

TABLE 2.—*Analysis of variance of site index for 56 stands classified according to figure 3*

Item	Degrees of freedom	Sum of squares	Variance
Variation explained by each factor of the classification:			
Topography.....	1	1,571	1,571*
Water table depth.....	1	1,341	1,341*
Texture.....	5	509	114*
Pore space.....	1	213	213*
Variation unexplained by the classification.....	47	902	19.2
Total.....	55	4,596	-----

\*Highly significant in comparison with unexplained variance.

This classification accounted also for the significance of the other factors listed in table 1, i. e., slope, phosphate in C horizon, soil type, and aspect. This was determined by subjecting the remainder variance of 19.2 to further examination for the purpose of determining whether the variations in growth rate which it represents were explained by relationships with any of these five factors. No significant reduction below 19.2 was obtainable, indicating that when the site classification of figure 2 was used, the other site factors were without significance. The expected site index for each stand was determined from figure 2. The deviations of these expected site indexes from the actual site indexes were calculated and compared with the remaining site factors. None of these factors accounted for the deviations. Apparently, factors not included in this study, such as stand history, caused these deviations.

It is worth while mentioning that the site factors of figure 2 explained almost entirely the variations in growth rate associated with soil series. There were four major soil series (11) among the stands, and, as indicated in table 1, their associated growth rates differed significantly. However, these differences in growth rate were accounted for largely by the differences between the soil types in topography, water-table depths, texture, and pore space.

## VEGETATION AS AN INDEX OF GROWTH RATE

The natural vegetation in the area where shipmast locust occurs on Long Island may be divided into two main types. In the more mesophytic forests, located principally on the moraines near the north shore, typical moisture-loving species, such as walnut, beech, and yellow poplar, occur. The less mesophytic vegetation is found principally on the relatively dry outwash plains south of the moraines. Typical species of this forest are white oak, chestnut oak, pin oak; and on the driest sites, pitch pine and scrub oak.

Study of the flora in the shipmast locust stands indicated that the presence of several tree and ground-cover species was associated with the growth rate of shipmast locust. On sites where yellow poplar (*Liriodendron tulipifera* L.) and celandine (*Chelidonium majus* L.) were present, shipmast locust made its best growth. The average site index for shipmast locust on the four sites in which yellow poplar was found was 78 feet, and on the four sites in which celandine was found, 74 feet. These site indexes were well above the average of 66 feet for all the stands studied. Of the seven stands in which these two species occurred, six were classed as hillside stands and one as flat terrain with a water table less than 15 feet deep. This classification indicated that favorable soil moisture conditions existed in these sites.

Other species which appear to have some value for indexing growth rate of shipmast locust on Long Island were sassafras (*Sassafras variifolium* (Salisb.) Ktze.) and white oak (*Quercus alba* L.). These species occurred in stands where the growth of shipmast locust ranged from average to poor. The average site index of the 6 stands in which sassafras occurred was 63 feet, and that of the 6 stands in which white oak occurred, 59 feet. Most of the other 80 associated species of trees, shrubs, and ground-cover plants were either in insufficient quantity or were distributed over too wide a range to indicate any relationship with the growth of shipmast locust.

Since the species mentioned have generally recognized site requirements, their presence should be useful as an approximate check to supplement and confirm site evaluations made by means of the topographic and edaphic classification shown in figure 2.

## RELATION OF SITE TO VIGOR OF SHIPMAST LOCUST

A striking feature of the shipmast locust stands on Long Island is the frequency of partly or entirely dead crowns. The trees grow well for a number of years, and then the upper part of the crown dies, sometimes as much as a quarter or a half of its length. The size of the trees at the time top decadence starts varies with the site.

Data on crown vigor in relation to site were obtained in 76 shipmast locust stands. On the basis of the largest trees, each stand was classified as to vigor by the following standards:

Vigor class	Condition of crown
1	Tops of all trees healthy.
2	Tops of some or all trees partly dead.
3	Tops of all trees dead.

The site index for each stand was determined from measurements on smaller trees in which decadence had not yet set in. In 52 of the

stands, undergrowth of competing native trees and shrubs was present. The remaining 24 stands were clear of competing trees and shrubs, although a grass and herb ground cover was present in all stands.

The data (table 3) indicate that the more vigorous stands were associated with higher site index. On sites of similar quality, as shown by stands in the same site-index class, vigor declined as the trees became taller. The prevalence of crown decadence in shipmast locust on Long Island is indicated by approximately 60 percent of the stands having partly dead crowns.

TABLE 3.—*Average height of the tallest trees and site index for 76 stands classified according to crown vigor*

Site index	Vigor class 1 (15 stands)		Vigor class 2 (46 stands)		Vigor class 3 (15 stands)	
	Frequency	Average maximum height	Frequency	Average maximum height	Frequency	Average maximum height
	Number	Feet	Number	Feet	Number	Feet
80-89	4	75	2	96	-----	-----
70-79	7	47	15	69	2	82
60-69	4	40	18	60	4	76
50-59	-----	-----	11	43	5	49
40-49	-----	-----	-----	-----	2	52
30-39	-----	-----	-----	-----	2	32
Average site index	74		66		56	

As a result of this examination, figure 3 was constructed. It shows for Long Island the maximum height to which shipmast locust grows and the height at which the various degrees of decadence occur for a range of site indexes. To use this chart as a guide in selecting sites or for cultural operations, the site index is first estimated, either by judging the growth-producing qualities of the site before planting or by measuring growth rate of the shipmast locust trees a few years after planting. For any site index, the approximate maximum height attainable is shown by line D, and the vigor at different heights by lines A, B, and C. On poor sites, for example, where the site index was 40, no stands over approximately 50 feet were observed, and stands above 30 feet were completely dead in the top. Hence, complete decadence occurred on such sites before the trees were large enough to produce a good yield of fence posts. On areas where the site index was 80, the trees reached a maximum height of over 100 feet and a height of 75 feet or more before decadence began (line A). This size is great enough for the production not only of fence posts, but also of barn sills, planks, piling, or railroad ties.

One feature of the chart that requires a further word of explanation is the use of two division lines (lines B and C) to separate vigor classes 2 and 3. It was observed that in stands where undergrowth of shrubs and other trees was removed, the shipmast locust trees reached a greater height before becoming completely decadent than in stands of equal site index where undergrowth was present. This was shown by comparing different stands, as well as parts of the same stand where the owners had removed the undergrowth in one part and not in the

other. These observations indicate that shipmast locust on poor sites can be grown to a greater height before becoming severely decadent if the stand is cleared of undergrowth or if undergrowth is kept out of the plantation by appropriate cultural treatments.

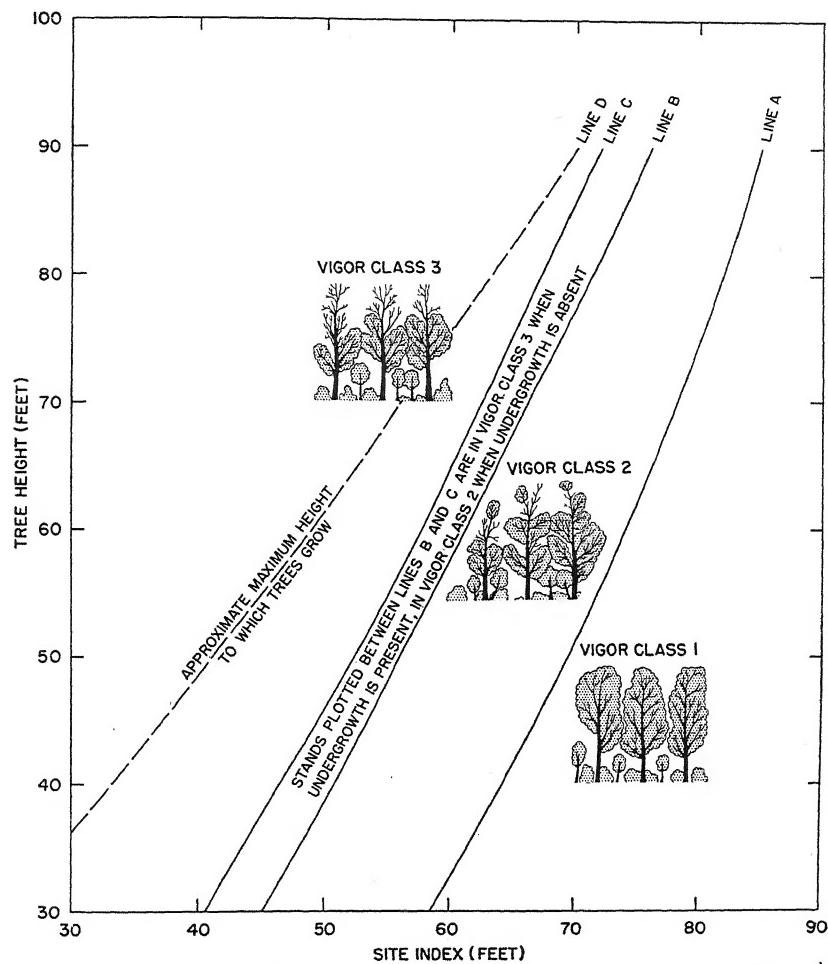


FIGURE 3.—Crown vigor of shipmast locust in relation to site index and tree height: Line A, start of crown decadence; line B, start of severe crown decadence in stands where undergrowth is present; line C, start of severe crown decadence in stands where undergrowth is removed; line D, maximum tree height.

The cause of decadence in shipmast locust is not definitely known. Examination of dead branches in the crowns showed the presence of borer holes, indicating that insects, probably *Cylleene robiniae* (Forst.), were one of the direct factors involved. Cope's observations (4) indicate that low winter temperature is a cause of crown decadence in shipmast locust. Whatever the direct cause of death, it is apparent from the data obtained in this study that predisposing factors are the

quality of the site, the height of the trees, and the undergrowth in the stand. As already shown, site quality in shipmast locust on Long Island is closely related to soil moisture supply. Undergrowth is important presumably because it also affects the amount of soil moisture available to the trees. It thus appears that a deficit in water supply may be the reason for top decadence.

A point to recall in this connection is that shipmast locust is not a native of Long Island, and therefore the crown development may not be entirely adapted to the existing soil moisture supply. This may be the reason why decadence increases as the trees grow in height.

An interesting illustration of the relation between tree height and vigor was observed in "topped" trees. Because of the unsightly appearance which decadent trees present, it has become common practice on landscaped areas to cut the dead tops out of such trees. Once the trees are topped, the lower part of the crown continues to live in a healthy condition. This observation suggested that the trees became decadent because they had reached a greater height than could be sustained permanently by their roots under the existing conditions.

#### GROWTH RATE OF SHIPMAST LOCUST AND COMMON BLACK LOCUST

The growth rate of shipmast locust in comparison with common black locust was determined from stand measurements over the same general area of Long Island. A total of 397 shipmast locust trees and 96 black locust trees were measured. The results are plotted in figure 4. Common black locust grew faster, on the average, than shipmast locust up to approximately 50 years. Thereafter, height growth of common black locust declined, while shipmast locust tended to continue its growth. The tallest shipmast locust trees measured were somewhat over 100 feet, which far surpassed the tallest common black locust in this area.

#### DISCUSSION

Since Long Island represents a specific combination of climatic, physiographic, and soil conditions (11, 17) that do not exist in exactly the same degree and relationship anywhere else, the specific values given above must be considered applicable only to the area studied. However, the practical importance of this study lies in the aid it may furnish for judging growth rate possibilities in places where shipmast locust is introduced outside Long Island. In this respect, therefore, the implications of these findings have significance beyond the exact numerical relationships presented.

Growth rate of established shipmast locust on Long Island appears to be influenced mainly by soil moisture supply and soil aeration. Two of the factors, namely, topography and depth of the water table are fundamentally indicative of moisture supply. The best growth was obtained in stands having favorable conditions of moisture supply and loose-structured soil. Poor growth occurred on flat or rolling terrain where the water table was deeper than 15 feet, even when the soil was not compact. The importance of water supply was shown also by the recognized requirements of the vegetation found to be indicative of site quality for shipmast locust. Some of the good sites for shipmast locust were vegetated naturally by yellow poplar and

celandine. The poor sites were represented for the most part by the flat so-called "sand plain" of Long Island, where white oak and sassafras were common. This type of site was usually associated with extreme decadence of shipmast locust, as indicated by dead tops in the crowns and failure of the trees to grow as tall as on other sites.

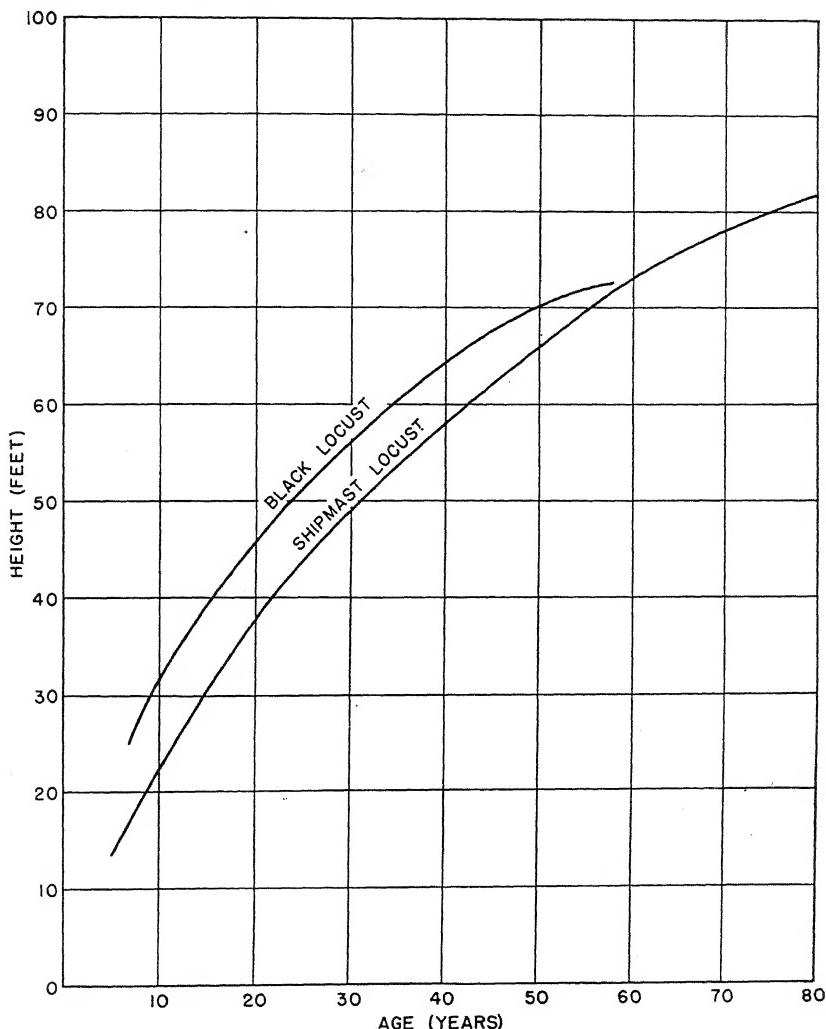


FIGURE 4.—Height growth of shipmast locust and common black locust on Long Island.

The trees measured in this study were in old established stands or sprout stands that came up voluntarily. These sites were all suitable for establishment and early survival. However, in selecting areas for planting, a wider range of conditions would be met. Some of these would be unsuitable even for initial establishment, while others would

be suitable for establishment but not for survival to maturity. Insofar as the site factors influencing establishment and early survival are the same as those that influence growth rate in mature trees, the general results of this study probably have some bearing on early growth in plantations.

Further light on this point is found in studies by Auten (1) and Grant (6) on the growth of common black locust. In these two studies, internal drainage of the soil was found to be the most significant factor in selecting sites for plantations. Soils with poor internal drainage were undesirable. In the present study, none of the stands were on poorly drained soils. The heaviest soil contained only 28 percent clay. Further, where moisture conditions were favorable, good internal drainage affected growth rate, as indicated by the relationships shown in figure 2.

Since the growth of shipmast locust was good where yellow poplar was present, a study by Auten (2) on the factors influencing growth rate of second-growth yellow poplar is noteworthy. He found topography and soil texture to be important. These are the same two factors that proved to be effective for indexing shipmast locust growth rate in this study. However, Auten's further division of topography according to aspect, exposure, and position did not hold for shipmast locust. The classification shown in figure 2 did not predict growth rate exactly, but variations from the predicted values were not related to aspect, exposure, position, or any other of the factors listed in table 1. This discrepancy may be due to the generally humid conditions on Long Island, or else shipmast locust is not as sensitive as yellow poplar to these location factors.

The better growth on hillsides having a drainage area above the stand was attributable apparently to better soil moisture conditions. This was indicated by the frequency of springs and seep spots on many of these sites. On some of the sites where surface water outlets were not observed, wells were present, and the water in these wells stood but a few feet below the ground surface. The manner in which a hillside topography brings about these soil moisture conditions, even when bedrock is absent, is shown by the studies of Norling (12) and Warington (20).

In view of the tendency to early top decadence on poor sites, it would be well for planters to consider both figures 2 and 3 in selecting sites. If we arbitrarily set 50 feet as the minimum height of trees for economic fence-post production, and 75 feet as the minimum for production of larger timbers, reference to figure 3 indicates site-index requirements of approximately 60 and 75, respectively. Now turning to figure 2, we see that, for Long Island conditions, sites located where the water table was deeper than 15 feet would generally be unsuitable for fence-post production, because such sites have indexes usually below 60. For production of large timbers, only hillsides with a moderately high sand concentration in the C horizon would be promising sites.

## SUMMARY

Based on site and growth measurements in 56 stands, a method of classifying site productivity for shipmast locust on Long Island, N. Y., was developed in which the following four factors were used: (1) Topography; (2) depth of water table; (3) soil texture; (4) soil pore space. This classification accounted for approximately 80 percent of the total variation in growth rate among the 56 stands. When these 4 factors were used, none of the other 35 site factors measured in this study had any apparent influence on growth rate.

Best growth occurred on hillsides and on flat terrain where the water table was between 4 and 15 feet deep, provided the soil was well aerated but not excessively sandy. Flat terrain where the water table was deeper than 15 feet, waterlogged soil, and heavy, poorly aerated soil were relatively unfavorable. The most favorable sites were those capable of supporting native growth of yellow poplar and celandine.

The height to which shipmast locust grew on Long Island varied with the quality of the site. On the best sites, shipmast locust attained a height of 100 feet or more, but on poor sites, the upper part of the crown died and height growth ceased. A chart is given which shows the maximum height to which shipmast locust grows and the heights at which various degrees of decadence occur for a range of site indexes on Long Island. Stands kept clear of competing tall vegetation reached a greater height before the trees became decadent. The limitation that site imposes on the commercial products attainable can be taken into consideration in selecting planting areas through the use of this chart.

Comparison of 397 shipmast locust and 96 common black locust trees located within the same general area indicated that the former grows more slowly in height until approximately 50 years, but thereafter more rapidly.

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## ORIGIN OF THE SEED COATS IN GUAYULE<sup>1</sup>

By LOUIS C. ERICKSON, formerly *assistant plant physiologist*, and H. M. BENEDICT, *plant physiologist, Special Guayule Research Project, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture*

### INTRODUCTION

The delayed germination of seed of guayule (*Parthenium argentatum* A. Gray) is attributable to two important factors, namely, an embryo dormancy of approximately 2 months' duration and the interfering action of a seed coat (2).<sup>2</sup> After the period of embryo dormancy satisfactory germination has been obtained by treating the seed with sodium hypochlorite or other oxidizing agents such as hydrogen peroxide or nitric acid. Benedict and Robinson (2) found that it is the inner seed coat which delays germination, and when this is removed chemical treatment is unnecessary. They found further that puncturing the inner seed coat with a needle or placing the seed with an intact inner seed coat in a high partial pressure of oxygen would induce germination. These results indicate that sufficient oxygen for germination does not ordinarily reach the embryo. Whether the inner seed coat consumes oxygen or is nearly impermeable to it is not known.

Borthwick and Robbins (3, pp. 284-285) stated that for lettuce, another composite, "the oxygen requirements increase rapidly with an increase in temperature, such that an adequate supply fails to diffuse through the membrane at the higher temperatures." Thus, at 30° C. or above untreated lettuce seed fails to germinate in air unless the seed coat is removed. Davis (4) presented evidence to indicate that the seed coats of lettuce become less permeable to gases at higher temperatures. The findings of Thornton (10) do not indicate a simple impermeability of the membrane of lettuce seed, since a high partial pressure of carbon dioxide induced germination at temperatures which ordinarily inhibit it. Borthwick and Robbins (3) found that in lettuce the seed coat immediately investing the embryo is made up of one or two layers of thick-walled endosperm cells and the inner cell wall of the inner layer of cells in the integument. They were under the impression that it was this integumentary membrane which impeded oxygen diffusion to the embryo.

The present study of the origin of the seed coats in guayule was undertaken as a preliminary step in a further investigation of the interfering action of the seed coat on germination.

### MATERIALS AND METHODS

Eighteen guayule plants of strain 593 in 8-inch pots were transferred from outdoor tables to the greenhouse on February 23. Within 3 weeks the plants were flowering. Heads in various stages of development were killed and fixed in Randolph's modification of Navashin's

<sup>1</sup> Received for publication August 2, 1946.

<sup>2</sup> Italic numbers in parentheses refer to Literature Cited, p. 334.

fluid (?) for paraffin sectioning. Dehydration was done with tertiary butyl alcohol. Sections were stained with safranine (5) and gentian violet dissolved in clove oil. Buds in young stages were fixed without dissection, but only the ray flowers were used in older stages. The mature seed was soaked in water for a few hours before dissection and fixation.

#### ANATOMY

The flowers of guayule were briefly described by Artschwager (1), and accounts of the morphology of reproduction were given by Kirkwood (9) and Esau (6). The present investigation was particularly concerned with the development of the anatropous ovule and the destiny of its cell layers.

A single massive integument forms in guayule, and at the stage when the megasporangium appears the integument has not yet completely enveloped the uniseriate nucellus (fig. 1, A). In many respects the ovule is similar to that of lettuce (8). The nucellus in guayule, however, is fused to the integument along most of its length; this condition is in marked contrast to that in lettuce, in which the nucellus is free from the integument almost to the chalazal end (8).

As in lettuce, the integument of guayule produces a nutritive jacket; this forms around all but the micropylar end of the embryo sac (fig. 1, B and C). It is derived from the inner cell layer of the integument and is a prominent feature during the development of the ovule. The nucellus, on the other hand, becomes crushed except on occasions such as those when a second embryo sac begins to form in one or more of its cells. Most of the early cell divisions in the nutritive jacket are anticlinal, but in later stages the jacket may become two cells or more thick. It is always thickest near the micropylar end of the ovule (fig. 2, A and B); it does not completely enclose this end, however, as it does the chalazal one.

The endosperm typically becomes cellular at an early stage in the development of the embryo (fig. 1, C), and soon it is composed of large and small cells. The large ones occupy a central position, whereas the small ones line the embryo sac. The endosperm tissue is most prominent at the micropylar end of the ovule, where it is persistent (fig. 2), but except for one or two layers of cells it is obliterated elsewhere as the embryo enlarges (fig. 3).

Destruction of intermediate layers of integumentary cells commences even before the embryo sac is mature; it progresses most rapidly at the chalazal end and permits most of the enlargement of the embryo sac to take place in this direction. Except for the outer cell layer of the integument and the vascular bundle, this ovuliferous structure is destroyed during the maturation of the seed (fig. 3). The last of these cell layers to be destroyed are the subepidermal ones and the nutritive jacket (fig. 3). The latter finally loses its densely protoplasmic appearance, a very characteristic feature retained during the development of the ovule and prior to maturation of the seed. During this final stage (fig. 3, B) the endosperm cells show signs of wall thickening at the outer corners.

The integumentary membrane of lettuce (3) is similar in appearance to a membrane found in guayule seed (figs. 2, B and C; 3, C). However, during the development of the guayule seed it was found that in

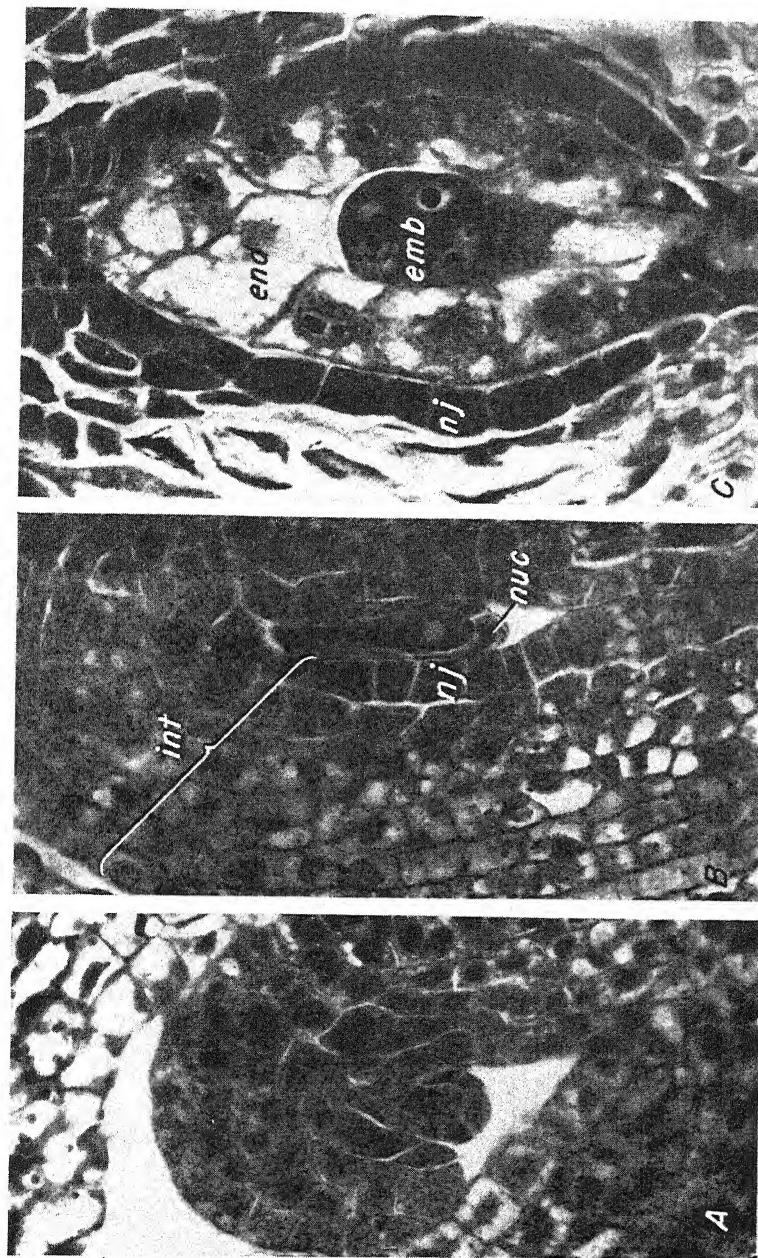


FIGURE 1.—Sections of guayule ovules: *A*, Young ovule; *B*, slightly older ovule showing origin of nutritive jacket and collapse of part of nucellus; *C*, ovule with young embryo. All  $\times 440$ . *emb*, Embryo; *end*, endosperm; *int*, integument; *nj*, nutritive jacket; *nuc*, nucellus.

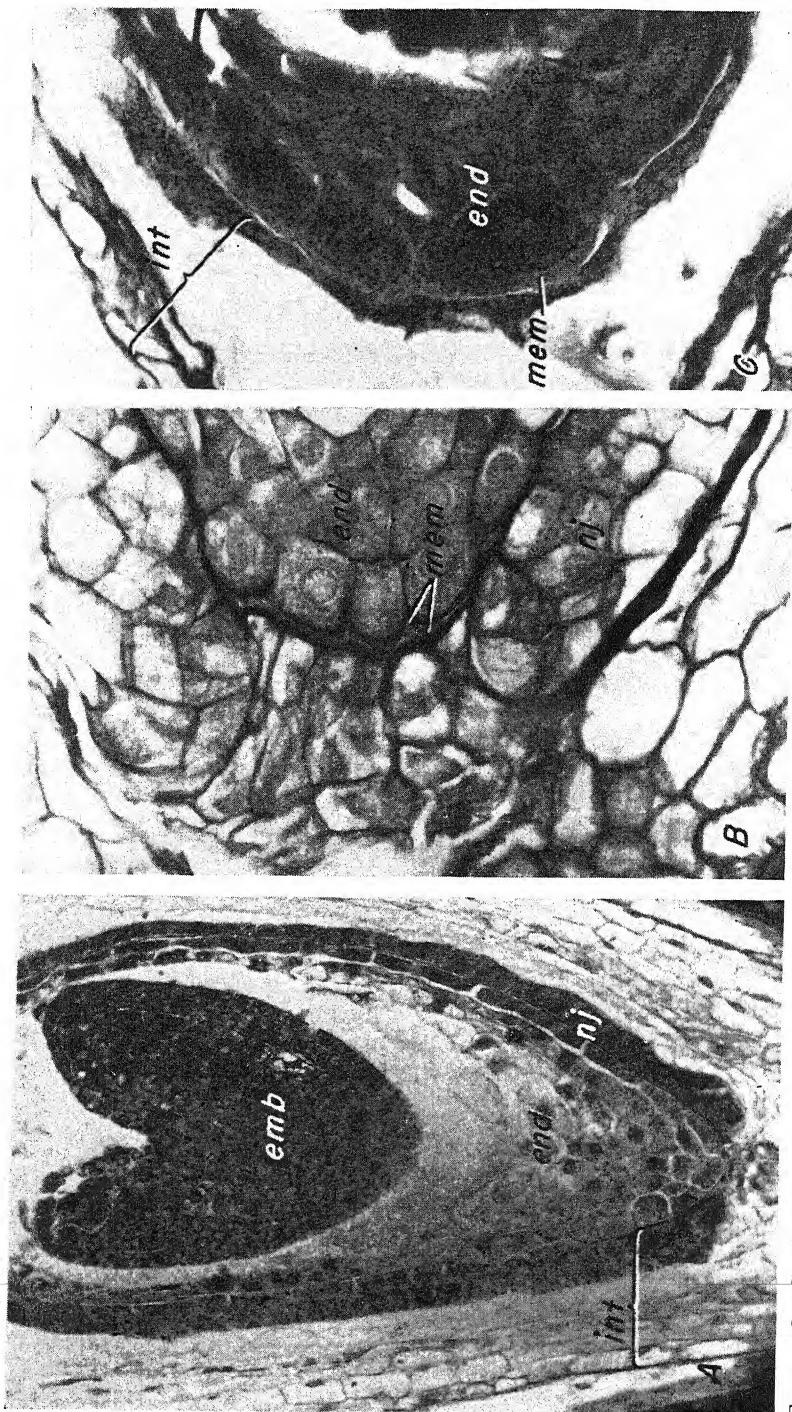


FIGURE 2.—Sections of guayule ovule and seeds. *A*, Ovule with immature embryo;  $\times 190$ . *B*, Micropylar end of immature seed, showing formation of membrane between endosperm and seed cells;  $\times 440$ . *C*, Micropylar end of mature seed;  $\times 490$ . *emb*, Embryo; *end*, endosperm; *int*, integument; *mem*, membrane; *nj*, nutritive jacket.

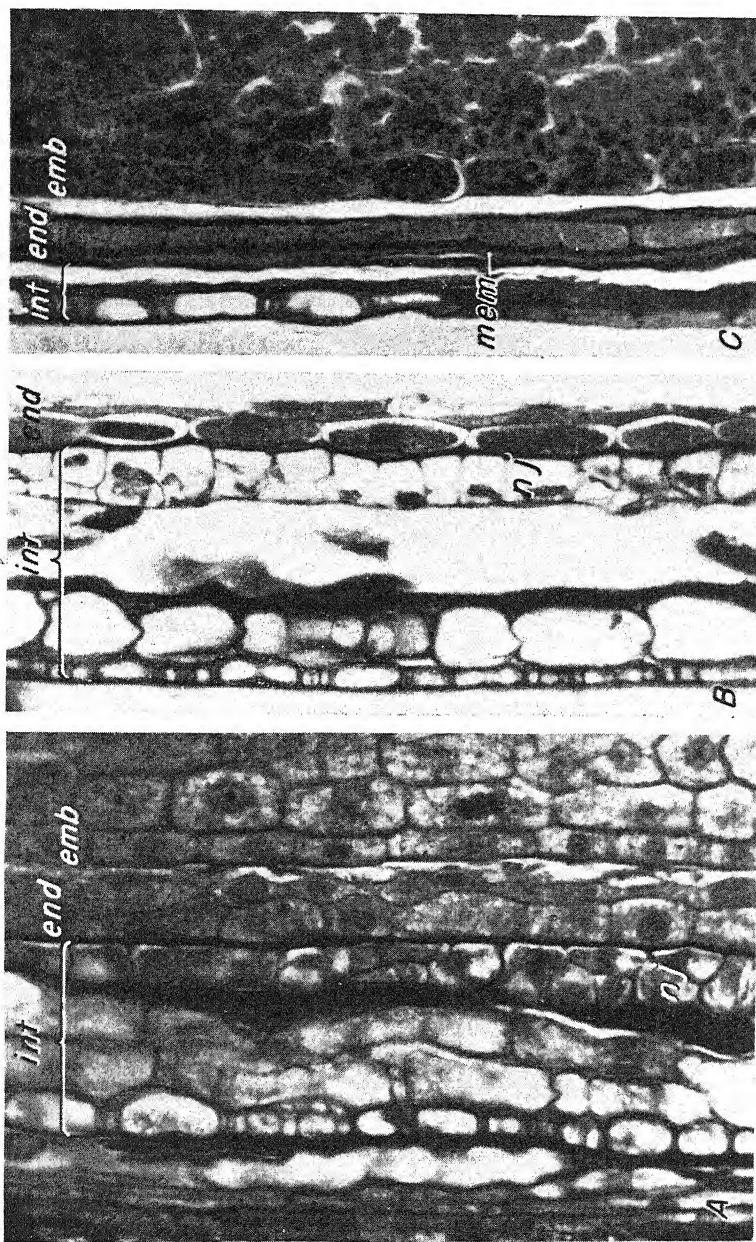


FIGURE 3.—Sections of guayule seeds; *A* and *B*, Immature seeds; *C*, mature seed. All  $\times 440$ . *int*, Integument; *end*, endosperm; *emb*, embryo; *mem*, membrane; *nj*, nutritive jacket.

the micropylar region where the nutritive jacket did not enclose all the endosperm cells a thick wall-like material was formed; this occupied positions even between endosperm cells (fig. 2, *B, mem.*). In view of the occurrence of this membrane where integumentary cells are lacking and also between certain endosperm cells, it appears that a so-called integumentary membrane as found in guayule, and perhaps in other composites, may in reality be a wall material excreted by the endosperm cells.

At the present time it has not been determined whether the dormancy in guayule seed is due to the membrane enclosing the endosperm or to the walls or living protoplasts of the endosperm cells.

After the pericarp of a mature guayule achene has been removed, the thin, white, soft, outer seed coat, which readily peels off, is the outer cell layer of the former integument. It is one cell thick except in the region where the vascular bundle adheres, and the cells are dead. The inner seed coat, which closely invests the embryo, is also thin and white but very tough. It is composed of a thick wall-like material and one or two cell layers of living endosperm cells. At the micropylar end of the seed the inner coat is somewhat thicker, because of the presence of endosperm tissue several cells in thickness.

#### SUMMARY

The achene of guayule contains a seed enclosed by two seed coats. The outer one is thin, white, and soft and is a single cell in thickness except where the vascular bundle occurs. It originates from the outer cell layer of the integument. The inner seed coat is thin, white, and tough and is composed of a membrane and one or two cell layers of living, thick-walled endosperm cells. At the micropylar end this seed coat is usually several cells thick. The membrane appears to be formed, at least in the micropylar region, by excretion of material from the endosperm cells.

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